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A61P 37/00 (2006.01)

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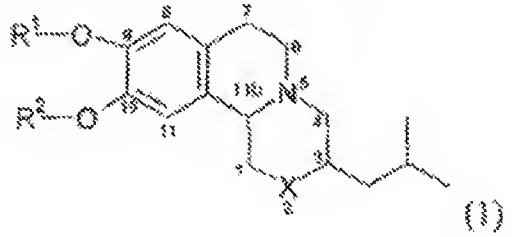
WO 2007/138365 A
Nuclear Medicine and Biology Vol. 33, No. 6, 2006, pages 685-694
Nuclear Medicine and Biology Vol. 24, No. 2, 1997, pages 197-199
Nuclear Medicine and Biology Vol. 21, No. 2, pages 151-156
Helvetica Chimica Acta Vol. 41, Issue 6, 1958, pages 1793-1806
Helvetica Chimica Acta Vol. 41, Issue 1, 1958, pages 119-139
Chirality Vol. 9, Issue 1, 1997, pages 59-62

(58) Field of Search:
INT Cl. A61K, C07D
Other: Online: CAS-ONLINE, EPODOC, WPI

(54) Abstract Title: **Desmethyl derivatives of tetrabenazine and pharmaceutical compositions thereof**

(57) Pharmaceutical compositions comprising a compound of the formula (1):



(1)

or a pharmaceutically acceptable salt or tautomer thereof and a pharmaceutically acceptable carrier and compounds of formula (1) per se or pharmaceutically acceptable salts or tautomers thereof, excluding certain isomers. R^1 and R^2 are each hydrogen or methyl, provided at least one of R^1 and R^2 is hydrogen, and X is CHOH or C=O. The compounds are 1,3,4,6,7,11b-hexahydrobenzo(a)quinolizine compounds, more specifically desmethyl derivatives of tetrabenazine, and may be useful in treating various diseases, in particular movement disorders such as Huntington's disease or Tourette's syndrome, depression, inflammatory disease, asthma, multiple sclerosis or an autoimmune myelitis, psychoses, cognitive deficit, schizophrenia, anxiety, dementia or diabetes mellitus.

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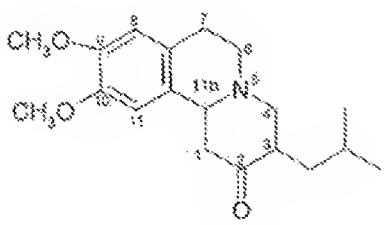
PHARMACEUTICAL COMPOUNDS

This invention relates to novel substituted 1,3,4,6,7,11b-hexahydro-benzo(a)quinolizine compounds, pharmaceutical compositions containing them, processes for making them and their therapeutic uses.

Background of the Invention

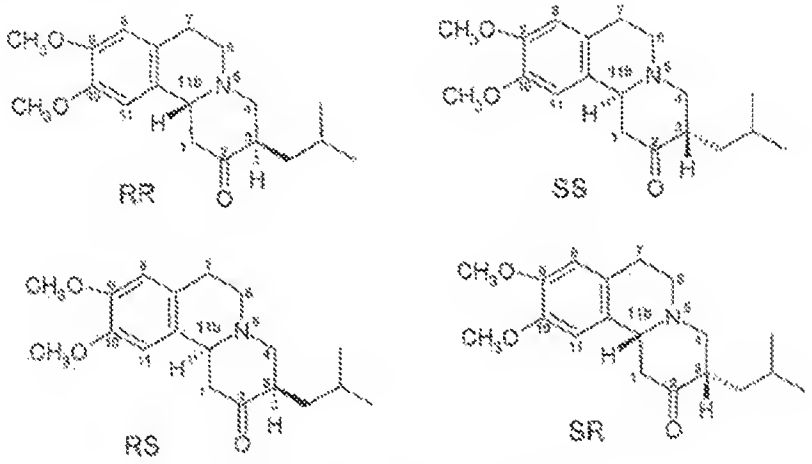
Tetrabenazine (Chemical name: 1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-3-(2-methylpropyl)-2H-benzo(a)quinolizin-2-one) has been in use as a pharmaceutical drug since the late 1950s. Initially used as an anti-psychotic, tetrabenazine is currently used for treating hyperkinetic movement disorders such as Huntington's disease, hemiballismus, senile chorea, tic, tardive dyskinesia, dystonia, myoclonus and Tourette's syndrome, see for example Ondo *et al.*, *Am. J. Psychiatry.* (1999) Aug; 156(8):1279-81 and Jankovic *et al.*, *Neurology* (1997) Feb; 48(2):358-62. The primary pharmacological action of tetrabenazine is to reduce the supply of monoamines (e.g. dopamine, serotonin, and norepinephrine) in the central nervous system by inhibiting the human vesicular monoamine transporter isoform 2 (hVMAT2). The drug also blocks postsynaptic dopamine receptors. Tetrabenazine is an effective and safe drug for the treatment of a variety of hyperkinetic movement disorders and, in contrast to typical neuroleptics, has not been demonstrated to cause tardive dyskinesia. Nevertheless, tetrabenazine does exhibit a number of dose-related side effects including causing depression, parkinsonism, drowsiness, nervousness or anxiety, insomnia and, in rare cases, neuroleptic malignant syndrome. The central effects of tetrabenazine closely resemble those of reserpine, but it differs from reserpine in that it lacks activity at the VMAT1 transporter. The lack of activity at the VMAT1 transporter means that tetrabenazine has less peripheral activity than reserpine and consequently does not produce VMAT1-related side effects such as hypotension.

The chemical structure of tetrabenazine is as shown below.



Structure of tetrabenazine

The compound has chiral centres at the 3 and 11b carbon atoms and hence can, theoretically, exist in a total of four isomeric forms, as shown below.



Possible tetrabenazine isomers

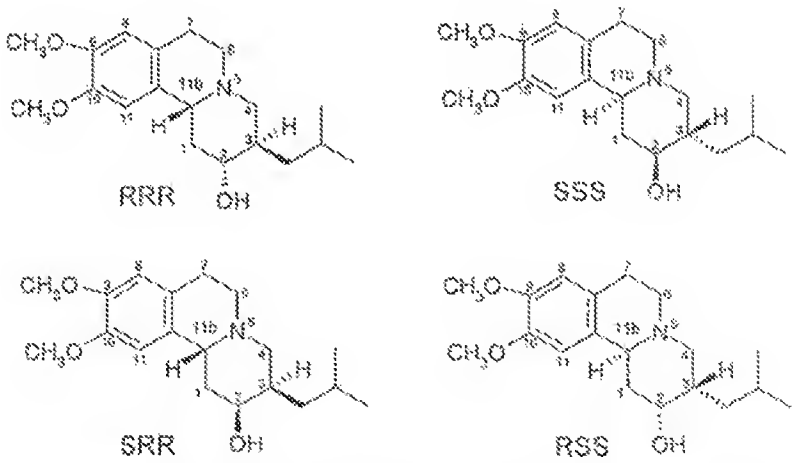
The stereochemistry of each isomer is defined using the "R and S" nomenclature developed by Cahn, Ingold and Prelog, see *Advanced Organic Chemistry* by Jerry March, 4th Edition, John Wiley & Sons, New York, 1992, pages 109-114. In the structures above and elsewhere in this patent application, the designations "R" or "S" are given in the order of the position numbers of the carbon atoms. Thus, for example, *RS* is a shorthand notation for 3*R*,11b*S*. Similarly, when three chiral centres are present, as in the dihydrotetrabenazines described below, the designations "*R*" or "*S*" are listed in the order of the carbon atoms 2, 3 and 11b. Thus, the 2*S*,3*R*,*11bR* isomer is referred to in short hand form as *SRR* and so on.

Commercially available tetrabenazine is a racemic mixture of the *RR* and *SS* isomers and it would appear that the *RR* and *SS* isomers (hereinafter referred to individually or collectively as *trans*-tetrabenazine because the hydrogen atoms at

the 3 and 11b positions have a *trans* relative orientation) are the most thermodynamically stable isomers.

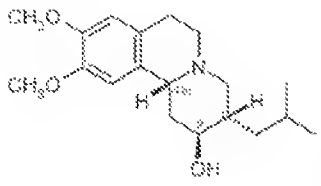
Tetrabenazine has somewhat poor and variable bioavailability. It is extensively metabolised by first-pass metabolism, and little or no unchanged tetrabenazine is typically detected in the urine. The major metabolite is dihydrotetrabenazine (Chemical name: 2-hydroxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-benzo(a)quinolizine) which is formed by reduction of the 2-keto group in tetrabenazine, and is believed to be primarily responsible for the activity of the drug (see Mehvar *et al.*, *Drug Metab.Disp*, 15, 250-255 (1987) and *J. Pharm. Sci.*, 76, No.6, 461-465 (1987)).

All eight dihydrotetrabenazine isomers have previously been identified and characterised. Four of the isomers are derived from the more stable *RR* and *SS* isomers of the parent tetrabenazine and have a *trans* relative orientation between the hydrogen atoms at the 3 and 11b positions) (see Kilbourn *et al.*, *Chirality*, 9:59-62 (1997) and Brossi *et al.*, *Helv. Chim. Acta.*, vol. XLI, No. 193, pp1793-1806 (1958). The structures of the four 3,11b *trans* dihydrotetrabenazine isomers are as shown below.

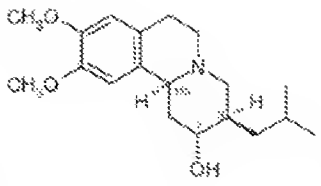


Structures of 3,11b *trans*-isomers of dihydrotetrabenazine

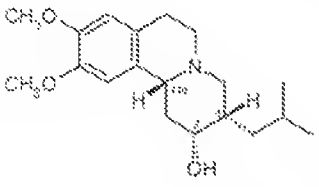
The four 3,11b *cis*-isomers are disclosed in our earlier patent applications WO2005/077946, WO2007/007105, WO2007/017643 and WO2007/017654. The structures of the 3,11b *cis* isomers are as follows:



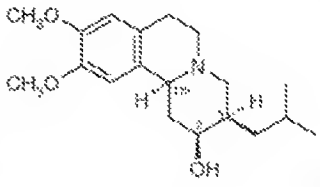
2*S*,3*S*,11b*R*



2*R*,3*R*,11b*S*



2*R*,3*S*,11b*R*



2*S*,3*R*,11b*S*

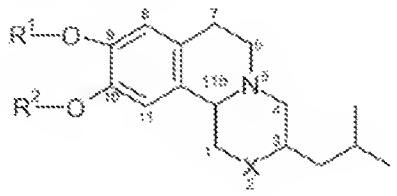
The above 3,11b *cis*-dihydrotetrabenazine isomers are disclosed as having a number of therapeutic uses including use in treating movement disorders (WO2005/077946), use in arresting or slowing the development of the symptoms of Huntington's disease (WO2007/007105), anti-inflammatory use (WO2007/017643), and anti-psychotic use (WO2007/017654).

The 9-desmethyl analogues of the *RRR* and *SSS* dihydrotetrabenazines described above are disclosed in Kilbourn *et al.*, *Chirality*, 9:59-62 (1997). The compounds were prepared as substrates for X-ray crystallographic studies for the purpose of determining the absolute structures of (+) α-dihydrotetrabenazine.

Summary of the Invention

The present invention relates to pharmaceutical uses of the des-methyl derivatives of the above tetrabenazines and dihydrotetrabenazines, and to novel des-methyl derivatives *per se*.

Accordingly, in a first aspect, the invention provides a pharmaceutical composition comprising a compound of the formula (1):

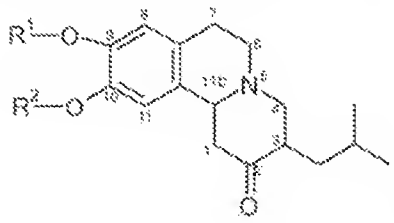


(1)

or a pharmaceutically acceptable salt or tautomer thereof;
wherein R^1 and R^2 are each selected from hydrogen and methyl, provided that at least one of R^1 and R^2 is hydrogen; and X is CHOH or C=O;
and a pharmaceutically acceptable carrier.

In a second aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt or tautomer thereof, as hereinbefore defined but excluding the compounds 2*R*, 3*R*, 11b*R* - 9-O-desmethyl-dihydrotetrabenazine and 2*S*, 3*S*, 11b*S* - 9-O-desmethyl-dihydrotetrabenazine.

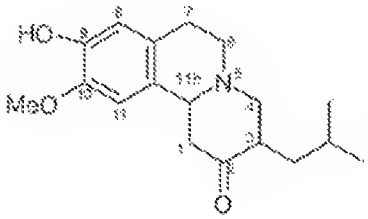
Within formula (1), one group of compounds can be represented by the formula (2):



(2)

and pharmaceutically acceptable salts thereof.

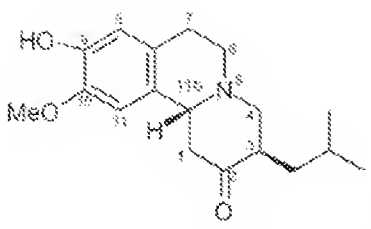
One particular sub-group of compounds within formula (2) is represented by formula (2a):

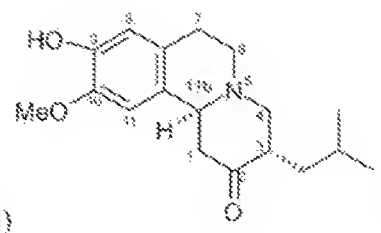


(2a)

and pharmaceutically acceptable salts thereof.

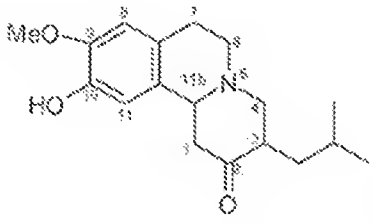
Within formula (2a), particular compounds are the compounds (2a-I) and (2a-II):


(2a-I)


(2a-II)

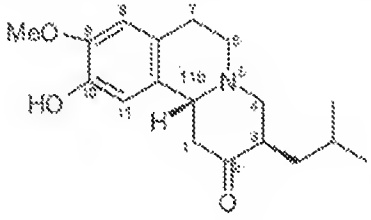
and pharmaceutically acceptable salts thereof.

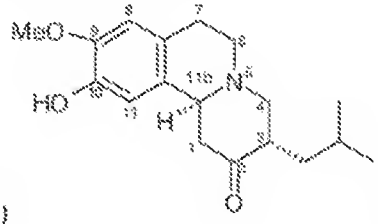
Another particular sub-group of compounds within formula (2) is represented by formula (2b):


(2b)

and pharmaceutically acceptable salts thereof.

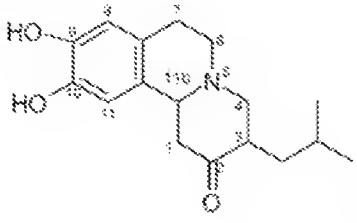
Within formula (2b), particular compounds are the compounds (2b-I) and (2b-II):


(2b-I)


(2b-II)

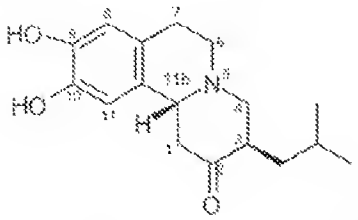
and pharmaceutically acceptable salts thereof.

Another particular sub-group of compounds within formula (2) is represented by formula (2c):

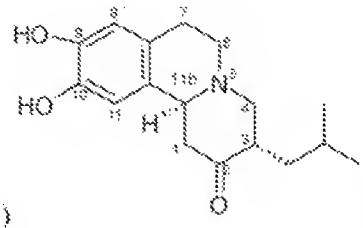

(2c)

and pharmaceutically acceptable salts thereof.

Within formula (2c), particular compounds are the compounds (2c-I) and (2c-II):



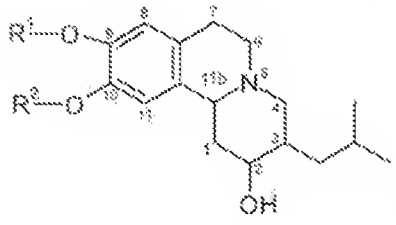
(2c-I)



(2c-II)

and pharmaceutically acceptable salts thereof.

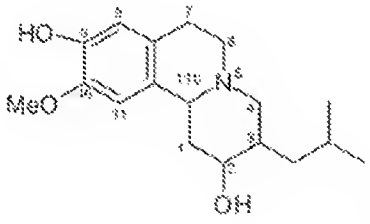
Another group of compounds within formula (I) is represented by formula (3):



(3)

and pharmaceutically acceptable salts thereof.

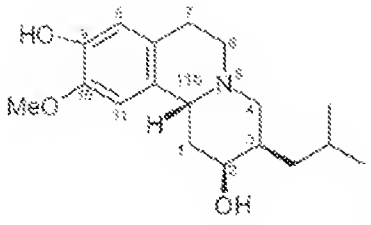
One particular sub-group of compounds within formula (3) is represented by formula (3a):

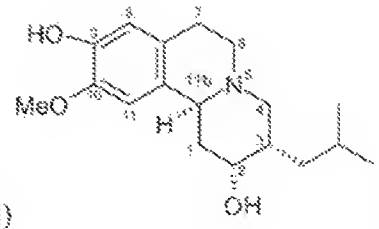


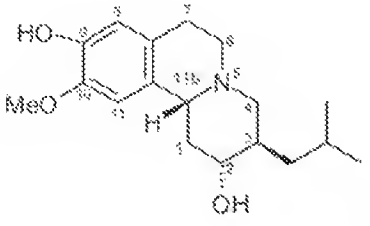
(3a)

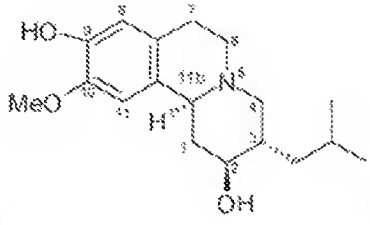
and pharmaceutically acceptable salts thereof.

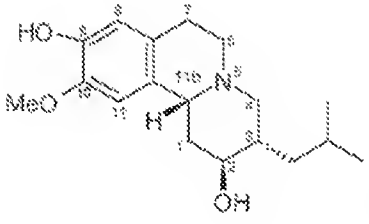
Within formula (3a), particular compounds are the compounds (3a-I), (3a-II), (3a-III), (3-a-IV), (3a-V), (3a-VI), (3a-VII) and (3a-VIII):

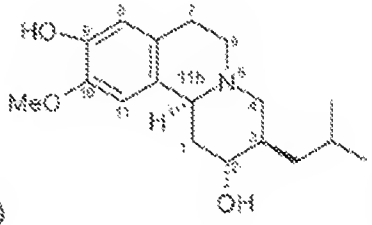

(3a-I)

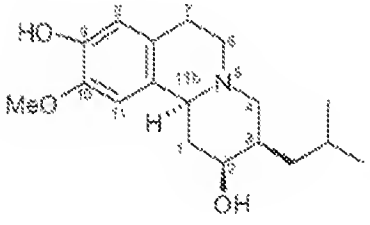

(3a-II)

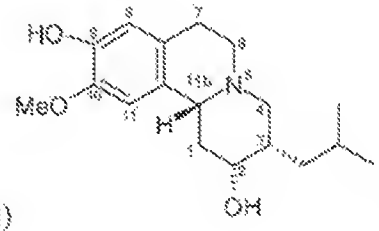

(3a-III)


(3a-IV)


(3a-V)


(3a-VI)


(3a-VII)


(3a-VIII)

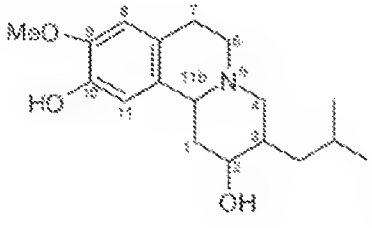
and pharmaceutically acceptable salts thereof.

Compounds (3a-III) and (3a-IV) are known compounds (see Kilbourn *et al.*, *Chirality*, 9:59-62 (1997)) and do not form part of the group of novel compounds *per se* of the invention.

Within formula (3a), one sub-group of compounds is constituted by compounds having a 3,11b-*trans*-stereochemical configuration, e.g. compounds (3a-I) and (3a-II).

Another sub-group of compounds is constituted by compounds having a 3,11b-*cis* stereochemical configuration, i.e. compounds (3a-V), (3a-VI), (3a-VII) and (3a-VIII).

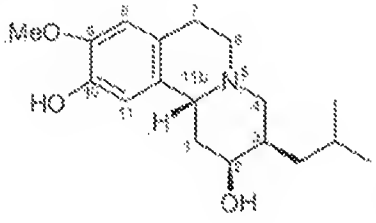
Another particular sub-group of compounds within formula (3) is represented by formula (3b):



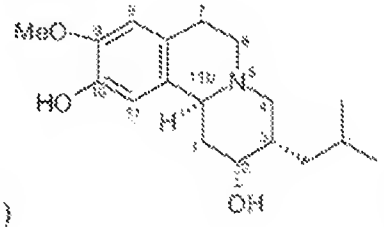
(3b)

and pharmaceutically acceptable salts thereof.

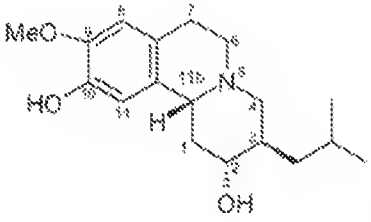
Within formula (3b), particular compounds are the compounds (3b-I), (3b-II), (3b-III), (3b-IV) (3b-V), (3b-VI) (3b-VII) and (3b-VIII):



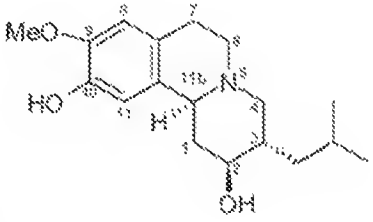
(3b-I)



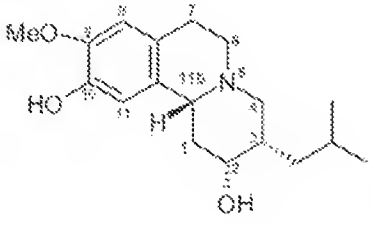
(3b-II)



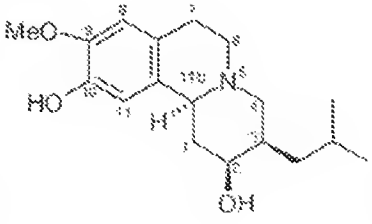
(3b-III)



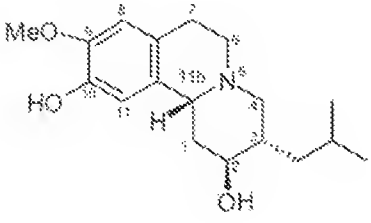
(3b-IV)



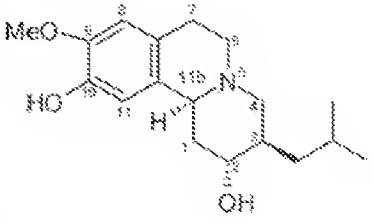
(3b-V)



(3b-VI)



(3b-VII)



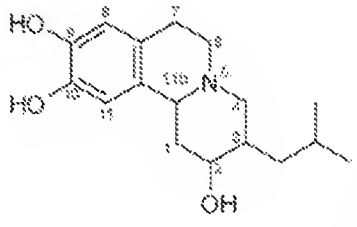
(3b-VIII)

and pharmaceutically acceptable salts thereof.

Within formula (3b), one sub-group of compounds is constituted by compounds having a 3,11b-*trans*-stereochemical configuration, i.e. compounds (3b-I), (3b-II), (3b-III) and (3b-IV).

Another sub-group of compounds is constituted by compounds having a 3,11b-*cis* stereochemical configuration, i.e. compounds (3b-V), (3b-VI), (3b-VII) and (3b-VIII).

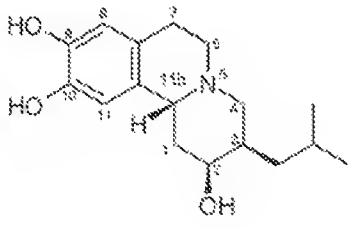
Another particular sub-group of compounds within formula (3) is represented by formula (3c):



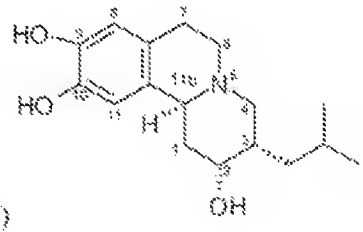
(3c)

and pharmaceutically acceptable salts thereof.

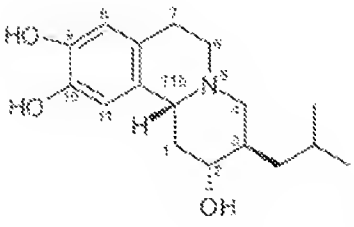
Within formula (3c), particular compounds are the compounds (3c-I), (3c-II), (3c-III), (3c-IV) (3c-V), (3c-VI) (3c-VII) and (3c-VIII):



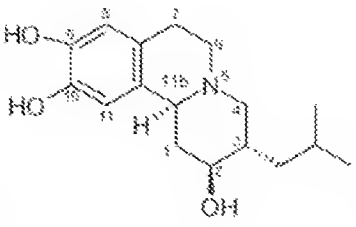
(3c-I)



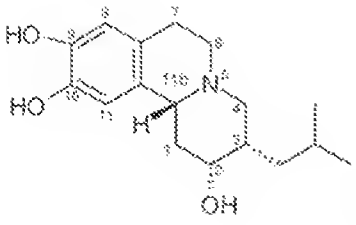
(3c-II)



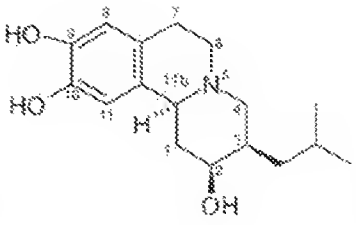
(3c-III)



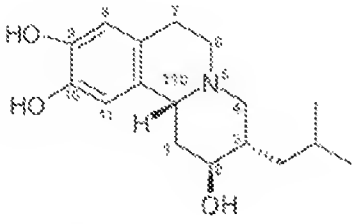
(3c-IV)



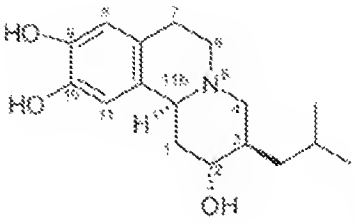
(3c-V)



(3c-VI)



(3c-VII)



(3c-VIII)

and pharmaceutically acceptable salts thereof.

Within formula (3c), one sub-group of compounds is constituted by compounds having a 3,11b-*trans*-stereochemical configuration, i.e. compounds (3c-I), (3c-II), (3c-III) and (3c-IV).

Another sub-group of compounds is constituted by compounds having a 3,11b-*cis* stereochemical configuration, i.e. compounds (3b-V), (3b-VI), (3b-VII) and (3b-VIII).

In this application, unless the context requires otherwise, a reference to a compound of the formula (1) includes not only formula (1) but also formulae (2), (2a), (2b), (2c), (3), (3a), (3b), (3c), subgroups thereof and particular compounds within the sub-groups as hereinbefore defined. The aforementioned compounds may also be referred to for convenience as the compounds of the invention.

Pharmaceutically Acceptable Salts

A reference to a compound of formula (1) also includes within its scope not only the free base of the compound but also its salts, and in particular acid addition salts.

Particular acids from which the acid addition salts are formed include acids having a pKa value of less than 3.5 and more usually less than 3. For example, the acid addition salts can be formed from an acid having a pKa in the range from +3.5 to -3.5.

Preferred acid addition salts include those formed with sulphonic acids such as methanesulphonic acid, ethanesulphonic acid, benzene sulphonic acid, toluene sulphonic acid, camphor sulphonic acid and naphthalene sulphonic acid.

One particular acid from which acid addition salts may be formed is methanesulphonic acid.

Salts can be prepared by the methods described herein or conventional chemical methods such as the methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free base form of the compound with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used.

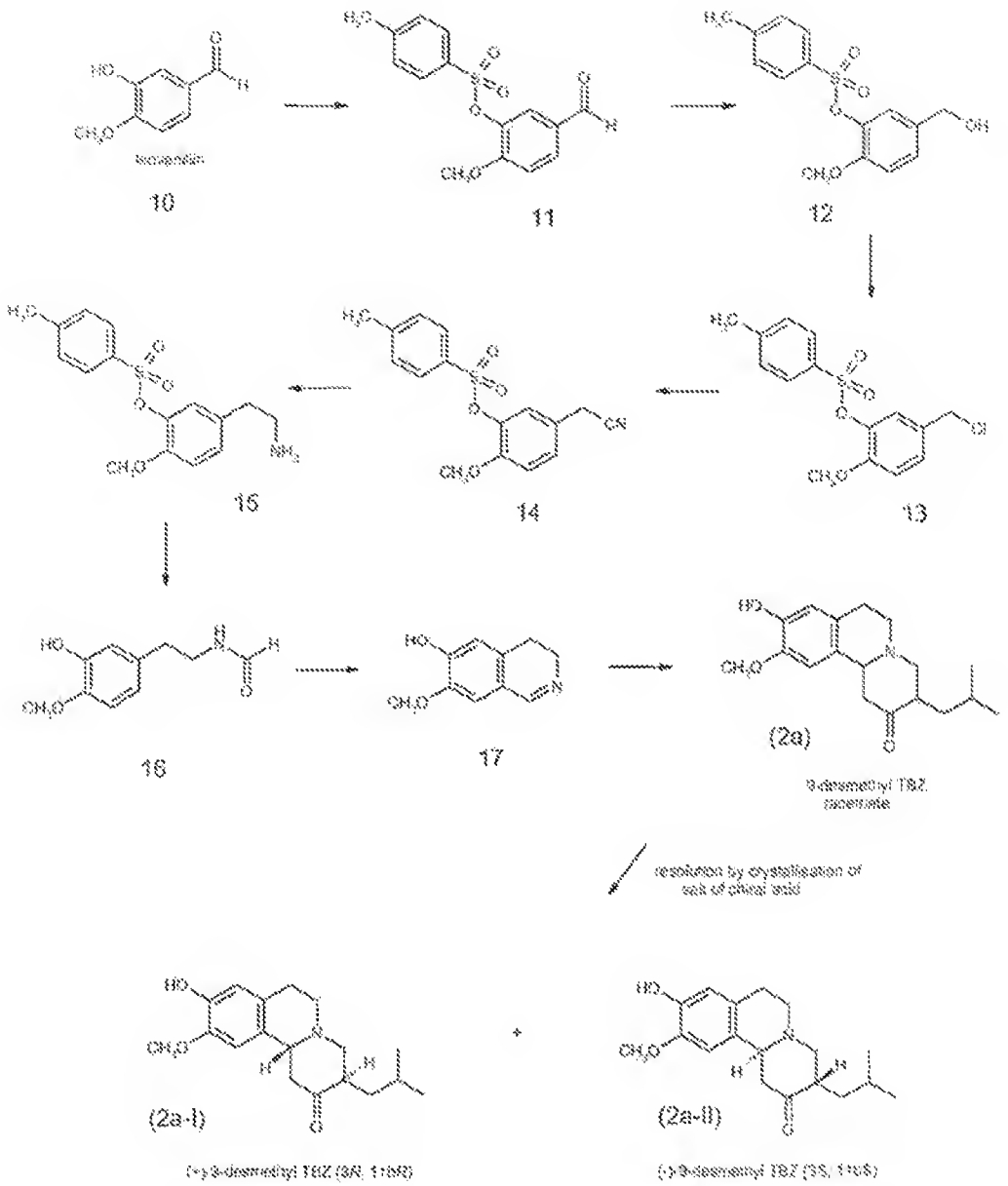
Salts may also be formed with a hydroxyl group on the benzene ring of the 1,3,4,6,7,11b-hexahydro-benzo(a)quinolizine structure. The salts can be formed by reaction of the compound with a base such as an alkali metal hydroxide (e.g. sodium hydroxide or potassium hydroxide) or alkaline earth metal hydroxide, ammonia or a primary secondary or tertiary amine.

The salts are typically pharmaceutically acceptable salts. However, salts that are not pharmaceutically acceptable may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salt forms also form part of the invention.

Methods for the Preparation of Compounds of the formula (1)

Compounds of the formula (1) can be prepared by the methods set out below or methods analogous thereto.

Compounds of the formula (1) wherein X is C=O, R^1 is hydrogen and R^2 is methyl (i.e. compounds of the formula (2a)) can be prepared by the sequence of reactions set out in Scheme 1.

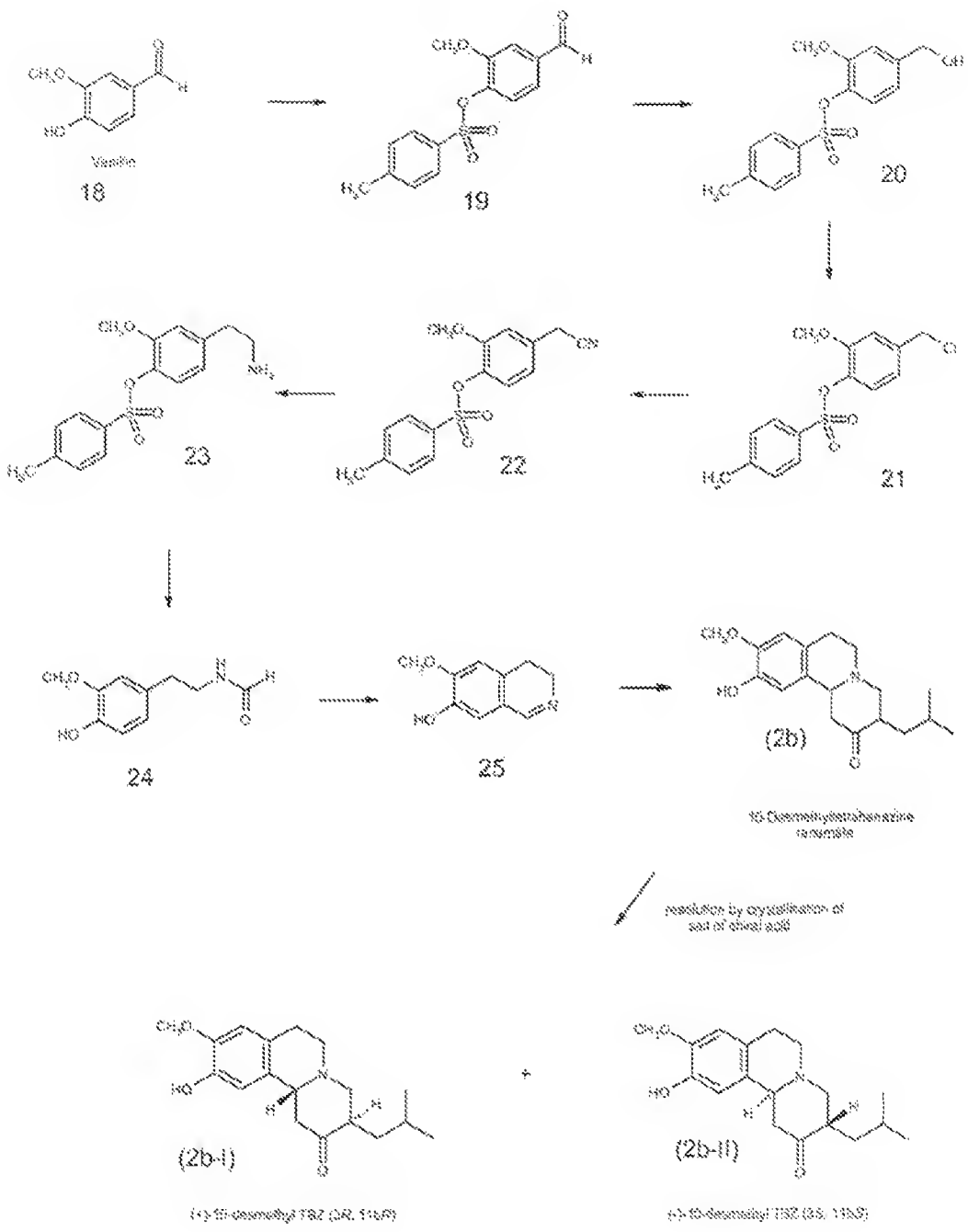


Scheme 1

In Scheme 1, isovanillin 10 is tosylated using tosyl chloride and pyridine in dichloromethane or another chlorinated solvent to give tosyl isovanillin 11. The tosyl vanillin 11 is then reduced to the corresponding alcohol 12 using sodium borohydride in a dichloromethane/methanol (10:1) solvent. Treatment of the alcohol 12 with neat thionyl chloride followed by heating to about 60 °C gives the benzyl chloride compound 13. The benzyl chloride compound 13 is then converted

to the nitrile 14 by reaction with potassium cyanide in acetonitrile in the presence of 18 crown 6 ether at reflux temperature. The nitrile is converted to the substituted phenylethylamine 15 by reduction using borane in tetrahydrofuran (THF). The tosyl protecting group on the oxygen atom attached to the *meta*-position on the substituted phenylethylamine 15 is removed by treatment with sodium hydroxide in ethanol/water (1:1) with heating to about 90 °C. The amino group of the substituted phenylethylamine 15 is then formylated by reaction with methyl formate at room temperature to give the N-formylphenylethylamine 16. Cyclisation of the N-formylphenylethylamine 16 is carried out under Bischler-Napieralski conditions using $POCl_3$ in acetonitrile to give the 3,4-dihydroisoquinoline 17. The 3,4-dihydroisoquinoline 17 is then reacted with 3-(N,N-dimethylaminomethyl)-5-methyl-2-hexanone in aqueous basic conditions (~ pH 8) at room temperature to give compound (2a) as a mixture of isomers. The individual isomers can be separated by chiral chromatography or forming a chiral salt with a chiral acid such as (+) or (-) camphorsulphonic acid followed by fractional recrystallisation from a solvent such as ethanol.

Compounds of the formula (1) wherein X is C=O, R^1 is methyl and R^2 is hydrogen (i.e. compounds of the formula (2b)) can be prepared by the sequence of reactions set out in Scheme 2.

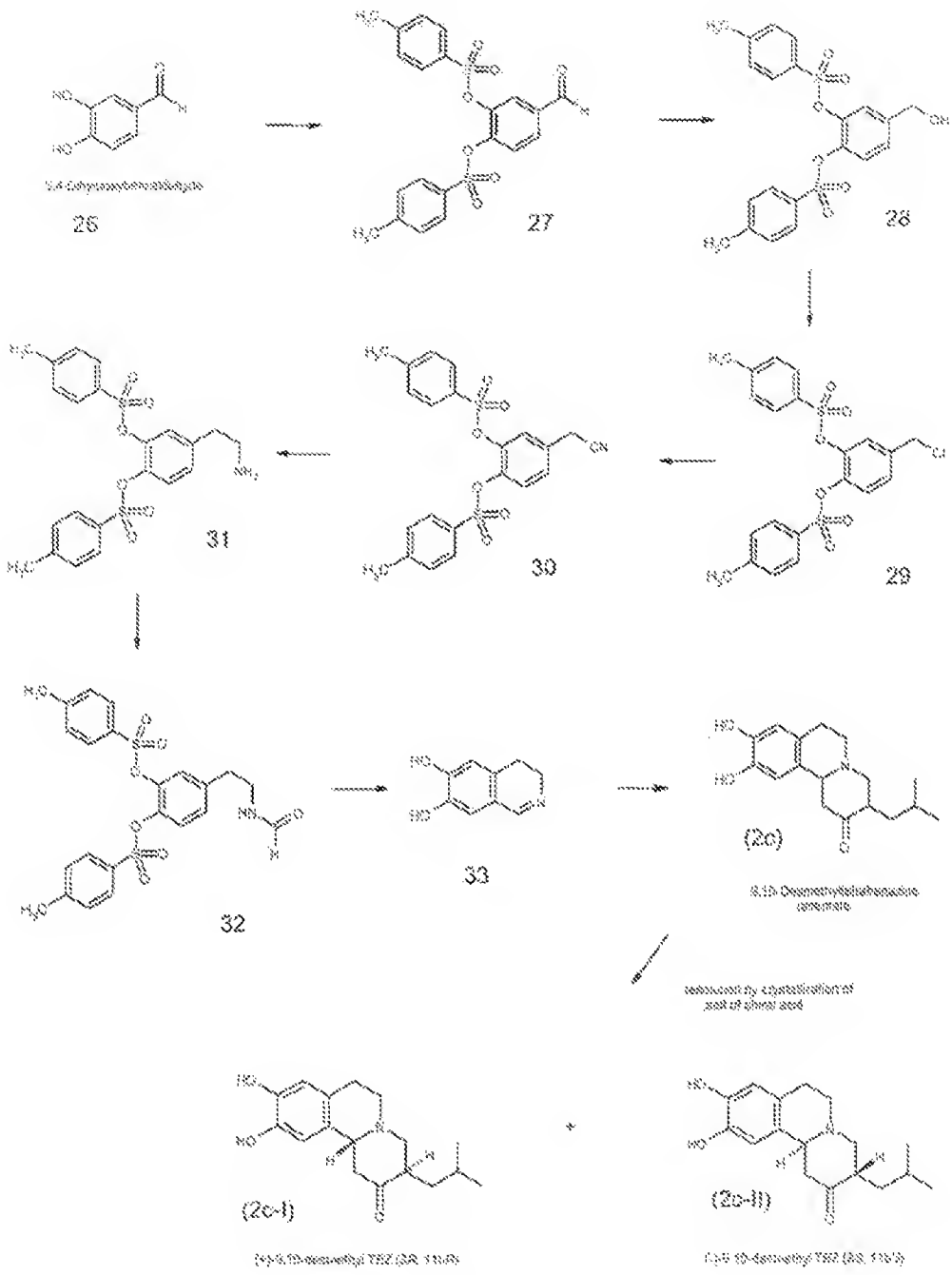


Scheme 2

The reaction conditions used in Scheme 2 are broadly the same as the conditions used in Scheme 1 except that the starting material for the synthesis is vanillin 18 rather than isovanillin 10. Thus the vanillin 18 is tosylated using tosyl chloride and pyridine in dichloromethane to give tosyl vanillin 19. The tosyl vanillin 19 is

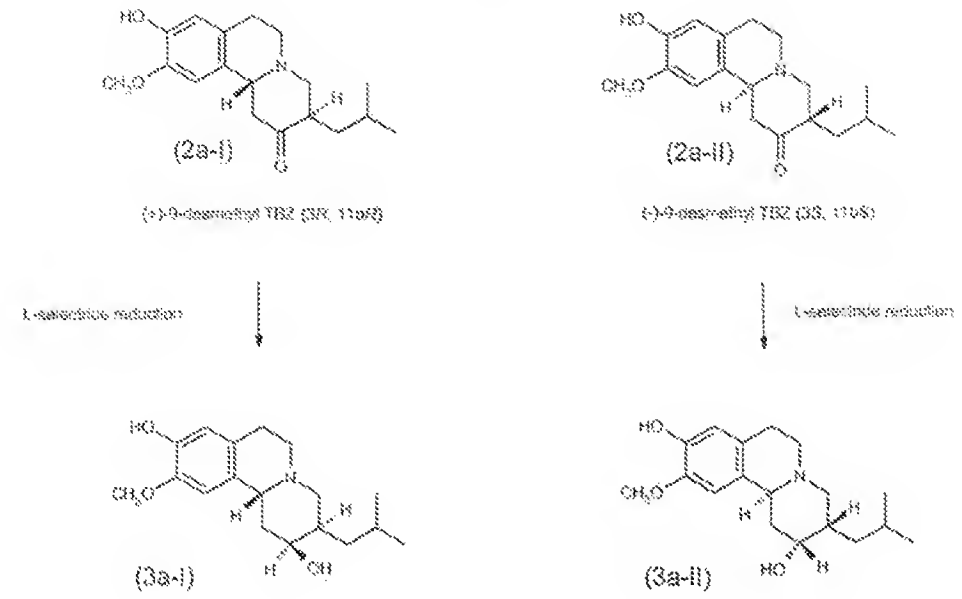
reduced to the corresponding benzyl alcohol 20 using sodium borohydride and the resulting benzyl alcohol 20 is converted to the benzyl chloride 21 by reaction with neat thionyl chloride. The benzyl chloride compound 21 is then converted to the nitrile 22 by reaction with potassium cyanide and the nitrile 22 in turn is converted to the substituted phenylethylamine 23 by reduction using borane. Removal of the tosyl protecting group from the substituted phenylethylamine 23 followed by formylation using methyl formate gives the N-formylphenylethylamine 24 which can then be cyclised using $POCl_3$ in acetonitrile to give the 3,4-dihydroisoquinoline 25. The 3,4-dihydroisoquinoline 25 is then reacted with 3-(N,N-dimethylaminomethyl)-5-methyl-2-hexanone to give compound (2b) as a mixture of isomers which can be separated into the individual isomers (2b-I) and (2b-II) by chiral chromatography or chiral salt formation followed by fractional recrystallisation.

Compounds of the formula (1) wherein *X* is C=O, and R^1 and R^2 are both hydrogen (i.e. compounds of the formula (2c)) can be prepared by the sequence of reactions set out in Scheme 3. The reaction conditions used in Scheme 3 are broadly similar to those employed in Schemes 1 and 2 as described above.



Scheme 3

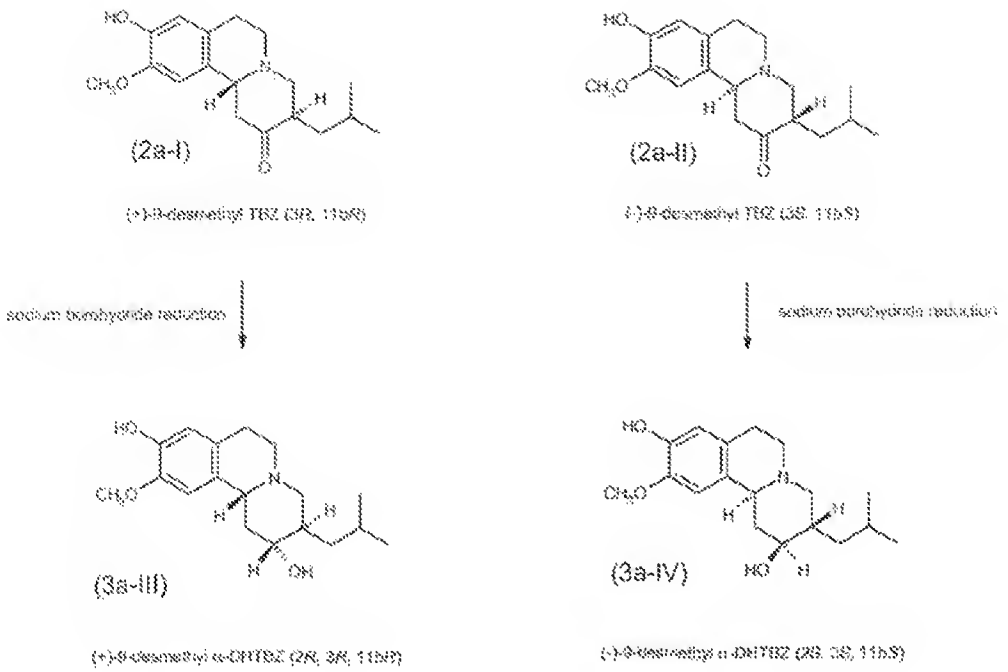
Compounds of the formulae (3A-I) and (3A-II) can be prepared by the synthetic routes shown in Scheme 4.



Scheme 4

In Scheme 4, the *RR* and *SS* isomers of 9-desmethyltetrabenazine are reduced to give the β-isomers (2,3-*cis* isomers) of 9-desmethyldihydrotetrabenazine by using the stereoselective reducing agent L-selectride.

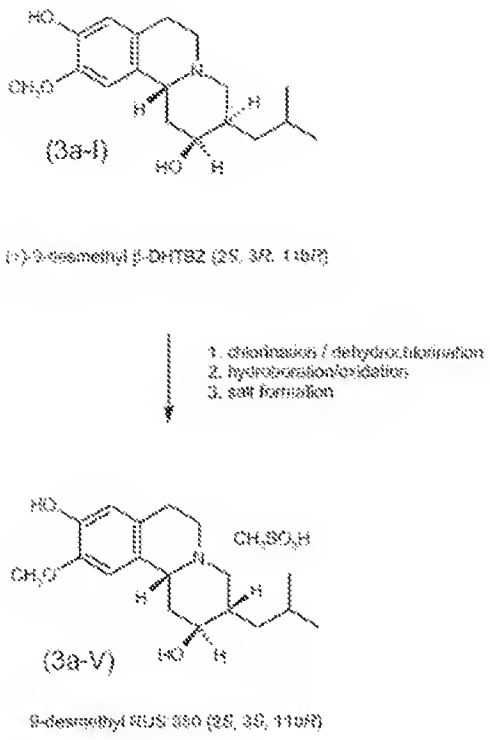
Compounds of the formulae (3a-III) and 3a-IV) can be prepared either by the methods described in Kilbourn *et al.*, *Chirality*, 9:59-62 (1997), or by the synthetic routes shown in Scheme 5.



Scheme 5

In Scheme 5, sodium borohydride is used instead of L-selectride and the 9-desmethyltetrabenazine isomers (2a-I) and (2a-II) are reduced to give the 9-desmethyldihydrotetrabenazine isomers (3a-III) and (3a-IV) respectively.

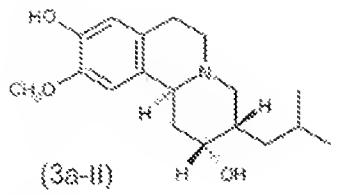
The compound of formula (3a-V) can be prepared by the route shown in Scheme 6.



Scheme 6

The starting material for Scheme 6 is compound (3a-I) which is prepared according the methods illustrated in Scheme 4. Compound (3a-I) is dehydrated to form an intermediate alkene (not shown) by chlorination/dehydrochlorination using phosphorus pentachloride in a chlorinated solvent such as dichloromethane followed by treatment with a base such as sodium carbonate. The intermediate alkene is then stereoselectively rehydrated by employing a hydroboration/oxidation procedure using borane-THF in tetrahydrofuran (THF) to form a borane intermediate (not shown) which is then oxidised with hydrogen peroxide in the presence of a base such as sodium hydroxide. The chlorination/ dehydrochlorination and hydroboration/oxidation reactions may be carried out using conditions analogous to those described in the examples of our earlier application WO2005/077946, the contents of which are incorporated herein by reference.

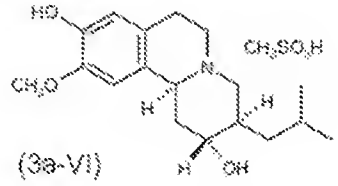
The compound of formula (3a-VI) can be prepared by the route shown in Scheme 7 wherein the chlorination/dehydrochlorination, and hydroboration/oxidation steps are carried out under the same general conditions as described in Scheme 6.



(3a-II)

(-)-9-desmethyl β-DHTBZ (2*R*, 3*S*, 11b*S*)

1. chlorination / dehydrochlorination
2. hydroboration/oxidation
3. salt formation



(3a-VI)

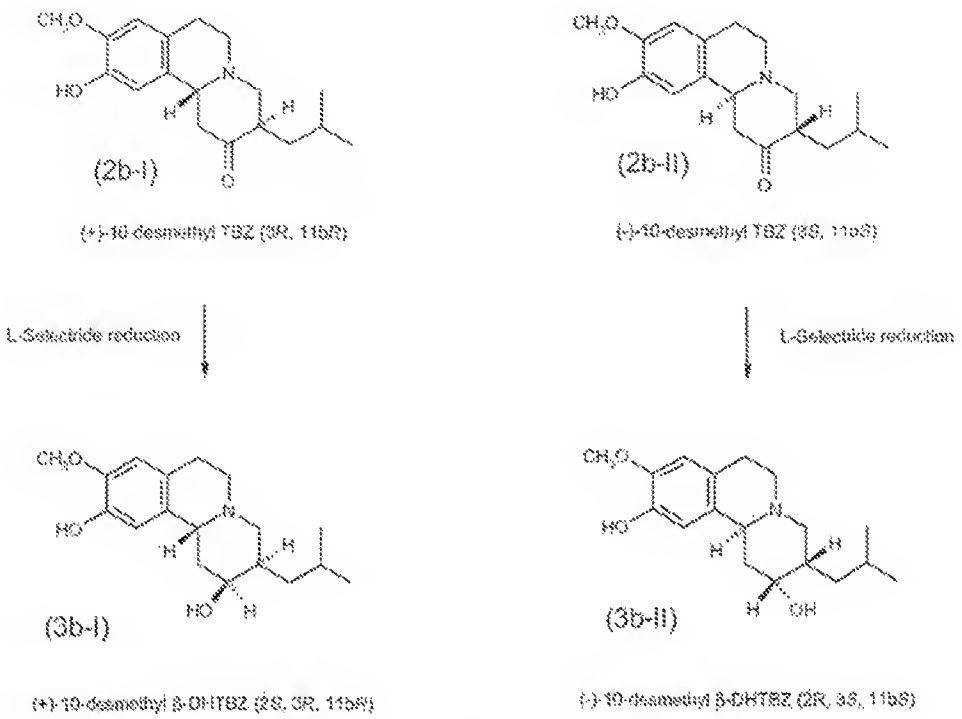
9-desmethyl RUS 351 (2*R*, 3*R*, 11b*S*)

Scheme 7

In a variation of the reaction sequence shown in Schemes 6 and 7, the intermediate alkene formed by dehydration of the compound of formulae (3a-I) and (3a-II) can be converted to an epoxide. The epoxidation reaction can be carried out using conditions and reagents well known to the skilled chemist, see for example J. March, *Advanced Organic Chemistry*, 4th Edition, John Wiley & Sons, New York, 1992, pages 826-829 and references therein. Typically, a per-acid such as *meta*-chloroperbenzoic acid (MCPBA), or a mixture of a per-acid and a further oxidising agent such as perchloric acid, may be used to bring about epoxidation.

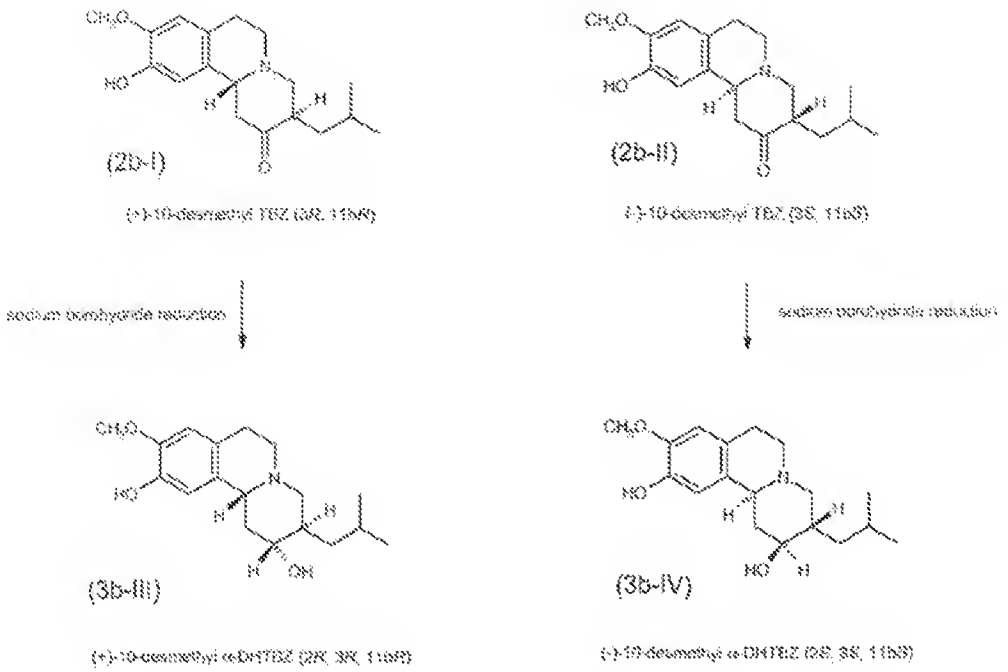
The epoxide can subsequently be subjected to ring opening by reaction with borane-THF in a polar non-protic solvent such as ether (e.g. tetrahydrofuran) at ambient temperature, followed by heating in the presence of water, sodium hydroxide and hydrogen peroxide at reflux temperature, to give the compounds (3a-VII) and (3a-VIII) wherein the 2- and 3-substituents have a *cis* relative configuration.

The compounds (3b-I) and (3b-II) can be prepared can be prepared by the L-selectride reduction of compounds (2b-I) and (2b-II) respectively shown in Scheme 8.



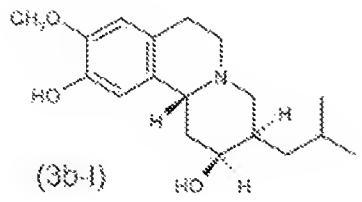
Scheme 8

The compounds (3b-III) and (3b-IV) can be prepared by the sodium borohydride reduction of compounds (2b-I) and (2b-II) respectively as shown in Scheme 9.



Scheme 9

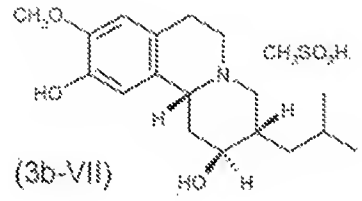
The compound (3b-VII) can be prepared from compound (3b-I) by a chlorination-dehydrochlorination-hydroboration-oxidation reaction sequence as shown in Scheme 10 using conditions analogous to those described in Scheme 6.



(3b-I)

(+)-10-desmethyl β-DHTBZ (2*S*, 3*R*, 11b*R*)

1. chlorination / dehydrochlorination
2. hydroboration/oxidation
3. salt formation

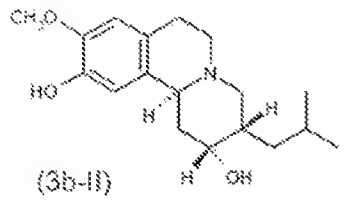


(3b-VII)

10-desmethyl RUS 350 (2*S*, 3*S*, 11b*R*)

Scheme 10

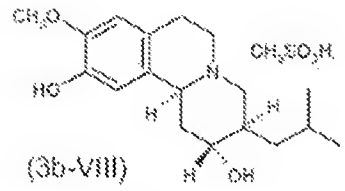
The compound (3b-VIII) can be prepared from compound (3b-II) by the chlorination-dehydrochlorination-hydroboration-oxidation reaction sequence shown in Scheme 11 using the conditions described in Scheme 6.



(3b-II)

(-)-10-desmethyl β-OHTBZ (2R, 3S, 11bS)

1. chlorination / dehydrochlorination
2. hydroboration/oxidation;
3. salt formation



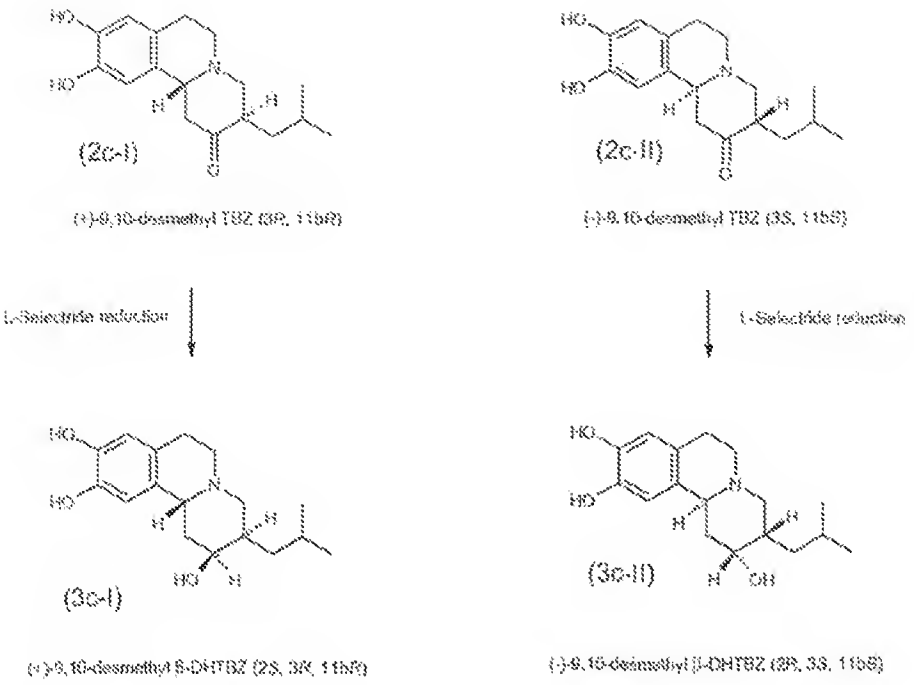
(3b-VIII)

10-desmethyl RUS 351 (2S, 3R, 11bS)

Scheme 11

The compounds (3b-V) and (3b-VI), in which the 2- and 3-substituents are in a *cis* relative configuration, can be prepared by subjecting the compounds (3b-I) and (3b-II) to chlorination/dehydrochlorination to give an intermediate alkene which is then oxidised to the epoxide and ring opened by treatment with borane, under the conditions described above for the preparation of (3a-VII) and (3a-VIII).

The compounds (3c-I) and (3c-II) can be prepared from compounds (2c-I) and (2c-II) respectively by reduction with L-selectride as shown in Scheme 12.



(+)-9,10-desmethyl TBZ (3R, 11bR)

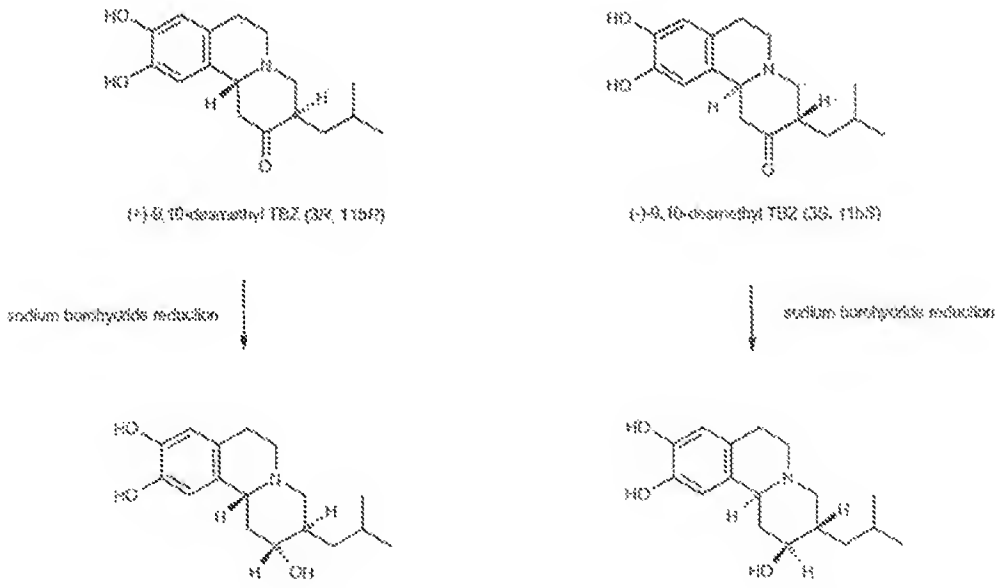
(-)-9,10-desmethyl TBZ (3S, 11bS)

(+)-9,10-desmethyl β-DHTBZ (2S, 3R, 11bR)

(-)-9,10-desmethyl β-DHTBZ (2R, 3S, 11bS)

Scheme 12

Compounds (3c-III) and (3c-IV) can be prepared from compounds (2c-I) and (2c-II) respectively by sodium borohydride reduction as shown in Scheme 13.



(+)-9,10-desmethyl TBZ (3R, 11bR)

(-)-9,10-desmethyl TBZ (3S, 11bS)

(+)-9,10-desmethyl α-DHTBZ (2R, 3R, 11bR)

(-)-9,10-desmethyl α-DHTBZ (2S, 3S, 11bS)

Scheme 13

The compounds (3c-V), (3c-VI), (3c-VII) and (3c-VIII), in which the hydrogen atoms at the 3 and 11b positions are in the *cis* relative orientation, can be prepared by the chlorination/dehydrochlorination procedures described above to give an intermediate alkene followed by either hydroboration/oxidation to give compounds (3c-VII) and (3c-VIII) or epoxidation/diborane treatment to give the compounds of formulae (3c-V) and (3c-VI).

In many of the reactions described above, it may be necessary to protect one or more groups to prevent reaction from taking place at an undesirable location on the molecule. Examples of protecting groups, and methods of protecting and deprotecting functional groups, can be found in *Protective Groups in Organic Synthesis* (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).
A hydroxy group may be protected, for example, as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc). In particular, it may be necessary or desirable to protect one or more of the hydroxyl groups present to prevent unwanted side reactions from taking place. For example, in the case of reactions involving compounds having hydroxyl groups at the 9- and 10-positions, particularly where the reactions involve oxidizing conditions, it may be desirable to protect the hydroxyl groups, e.g. example by forming an acetonide derivative, or a dibenzyl derivative or a mono- or di-tosyl derivative.

Once formed, the compounds of the invention can be purified by standard methods such as recrystallisation and chromatography.

Biological Properties and Therapeutic Uses

It is envisaged that the compounds of the invention will be useful in the prophylaxis or treatment of a variety of different disease states and conditions.

Accordingly, in another aspect, the invention provides a compound of the formula (1) as defined herein, or a pharmaceutically acceptable salt thereof, for use in medicine.

In another aspect, the invention provides a compound of the formula (1) as defined herein, or a pharmaceutically acceptable salt thereof, for use in the treatment of movement disorders.

The movement disorders can be, for example, hyperkinetic movement disorders such as Huntington's disease, hemiballismus, senile chorea, tic disorders, tardive dyskinesia, dystonia, myoclonus and Tourette's syndrome.

Of particular interest in the treatment of hyperkinetic movement disorders are the compounds 2a-I, 2b-I, 2c-I, 3a-I, 3b-I, 3c-I, 3a-V, 3b-VII and 3c-VII, or pharmaceutically acceptable salts thereof.

The compounds of the invention may be used to slow down or halt the progression of Huntington's disease, or to slow down or halt the development of the symptoms of Huntington's disease.

Of particular interest for use in slowing down or halting the progression of Huntington's disease, or slowing down or halting the development of the symptoms of Huntington's disease are the compounds 3a-V, 3b-VII and 3c-VII, or pharmaceutically acceptable salts thereof.

It is also envisaged that the compounds of the invention may be useful in the treatment of depression.

In a further aspect, the invention provides a compound of the formula (1) as defined herein, or a pharmaceutically acceptable salt thereof, for use in the treatment of inflammatory diseases.

Examples of inflammatory diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, traumatic arthritis, gouty arthritis, rubella arthritis, psoriatic arthritis, and other arthritic conditions; acute or chronic inflammatory disease states such as the inflammatory reaction induced by endotoxin or inflammatory bowel disease; Reiter's syndrome, gout, rheumatoid spondylitis, chronic pulmonary inflammatory disease (e.g. chronic obstructive pulmonary disease (COPD)), Crohn's disease and ulcerative colitis.

Particular inflammatory diseases and conditions are those that are sensitive to sigma receptor ligands, for example, sigma receptor antagonists.

One particular inflammatory disease is rheumatoid arthritis.

In another aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for treating, or a pharmaceutically acceptable salt thereof, for use in treating asthma.

In another aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for use in treating multiple sclerosis or an autoimmune myelitis.

The treatment of multiple sclerosis may consist of or comprise any one or more of:

- halting the progression of the disease;
- slowing the progression of the disease;
- modifying the progression of the disease;
- providing symptomatic relief, e.g. by eliminating or reducing the severity of one or more symptoms;
- extending periods of remission;
- preventing relapses;
- reducing the severity of relapses; and
- preventing or slowing the progression from an initial period of relapsing-remitting MS to secondary progressive MS.

The treatment may consist of or comprise the elimination, amelioration or reduction in severity of any one or more symptoms of multiple sclerosis, in any combination, selected from:

- weakness and/or numbness in one or more extremities;
- tingling of the extremities;
- tight band-like sensations around the trunk or limbs;
- tremor of one or more extremities;
- dragging or poor control of one or both legs;
- spastic or ataxic paraparesis;
- paralysis of one or more extremities;

- hyperactive tendon reflexes;
- disappearance of abdominal reflexes;
- Lhermitte's sign;
- retrobulbar or optic neuritis;
- unsteadiness in walking;
- problems with balance,
- increased muscle fatigue;
- brain stem symptoms (diplopia, vertigo, vomiting);
- disorders of micturition;
- hemiplegia;
- trigeminal neuralgia;
- other pain syndromes;
- nystagmus and ataxia;
- cerebellar-type ataxia;
- Charcot's triad; diplopia;
- bilateral internuclear ophthalmoplegia;
- myokymia or paralysis of facial muscles;
- deafness;
- tinnitus;
- unformed auditory hallucinations (because of involvement of cochlear connections);
- transient facial anesthesia or of trigeminal neuralgia;
- urinary and/or faecal incontinence
- bladder dysfunction euphoria;
- depression;
- fatigue;
- dementia;
- dull, aching pain in the low back;
- sharp, burning, poorly localized pains in a limb;
- abrupt attacks of neurologic deficit;
- dysarthria and ataxia;
- paroxysmal pain and dysesthesia in a limb;
- flashing lights;

- paroxysmal itching;
- tonic seizures;
- changes in sensation;
- visual problems;
- muscle weakness;
- difficulties with coordination and speech;
- cognitive impairment;
- overheating; and
- impaired mobility and disability.

Compounds of particular interest in the treatment of inflammatory diseases, multiple sclerosis and asthma are compounds 3a-V, 3b-VII and 3c-VII, or pharmaceutically acceptable salts thereof.

The invention also provides a compound of the formula (I) as defined herein, or a pharmaceutically acceptable salt thereof, for use in the treatment of psychoses.

Thus, the compounds of the invention may be used to prevent, alleviate or reduce any one or more psychotic episodes, psychoses or symptoms selected from:

- delusions;
- hallucinations;
- visual hallucinations;
- auditory hallucinations;
- hallucinations involving tactile sensations, tastes or smells;
- confusion;
- emotional, behavioral, or intellectual disturbances;
- withdrawal from reality;
- illogical and/or disorganized patterns of thinking;
- paranoid or delusional beliefs;
- paranoia
- grandiose delusions;
- persecutory or self-blaming delusions; and
- personality changes.

The psychotic episodes, psychoses or symptoms prevented, alleviated or reduced in accordance with the invention may be any one or more selected from those arising from or associated with:

- psychosis caused by or associated with schizophrenia;
- psychosis caused by or associated with bipolar disorder (manic depression);
- psychosis caused by or associated with severe clinical depression;
- psychosis induced by disorders and conditions such as:
    - electrolyte disorder;
    - urinary tract infections in the elderly;
    - pain syndromes;
    - drug toxicity;
    - drug withdrawal; and
    - infections of or injuries to the brain;
- psychosis caused by chronic psychological stress (brief reactive psychosis);
- psychosis triggered or exacerbated by severe mental stress; and
- psychosis triggered by or arising from or following illnesses and conditions such as AIDS, leprosy, malaria and mumps.

In one embodiment, the symptoms or psychoses arise from or are associated with schizophrenia and may be any one or more symptoms selected from:

- delusions;
- hallucinations;
- confusion;
- emotional, behavioral, or intellectual disturbances;
- withdrawal from reality; and
- illogical patterns of thinking.

In a further aspect, the invention provides a compound of the formula (1) as hereinbefore defined, or a pharmaceutically acceptable salt thereof, for use in treating a cognitive deficit associated with schizophrenia.

In another aspect, the invention provides a compound of the formula (1) as defined herein, or a pharmaceutically acceptable salt thereof, for use in treating anxiety.

In a further aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for treating a cognitive deficit in a patient.

In another aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for use in treating dementia.

The dementia may be, for example, dementia associated with Alzheimer's disease, or Lewy body dementia, or dementia arising from injury to the cerebrovascular system (e.g. stroke).

The compounds of the invention may be used to treat cognitive deficits in patients suffering from dementia.

Compounds of particular interest for the treatment of psychoses, dementia and cognitive deficits are the compounds 3a-VI, 3b-VIII and 3c-VIII, or pharmaceutically acceptable salts thereof.

In a further aspect, the invention provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for use in the treatment of diabetes mellitus.

The invention also provides a compound of the formula (1), or a pharmaceutically acceptable salt thereof, for use in (i) enhancing glucose dependent insulin secretion in a patient; and/or (ii) enhancing glucose tolerance in a patient.

The invention further provides the use of a compound of the formula (1), or a pharmaceutically acceptable salt thereof, as defined herein for the manufacture of a medicament for the prophylaxis or treatment of any one or more of the diseases or conditions listed above.

The invention also provides a method for the treatment of any one or more of the diseases or conditions listed above, which method comprises administering to a patient (e.g. a mammalian patient such as a human) a therapeutically effective (preferably non-toxic) amount of a compound of the formula (1) or a pharmaceutically acceptable salt thereof.

In treating each of the conditions listed above, the compounds of the invention will generally be administered to a subject in need of such administration, for example a human or animal patient, preferably a human.

The compounds will typically be administered in amounts that are therapeutically or prophylactically useful and which generally are non-toxic. However, in certain situations, the benefits of administering a compound of the invention may outweigh the disadvantages of any toxic effects or side effects, in which case it may be considered desirable to administer compounds in amounts that are associated with a degree of toxicity.

A typical daily dose of the compound can be in the range from 0.025 milligrams to 5 milligrams per kilogram of body weight, for example up to 3 milligrams per kilogram of bodyweight, and more typically 0.15 milligrams to 5 milligrams per kilogram of bodyweight although higher or lower doses may be administered where required.

By way of example, an initial starting dose of 12.5 mg may be administered 2 to 3 times a day. The dosage can be increased by 12.5 mg a day every 3 to 5 days until the maximal tolerated and effective dose is reached for the individual as determined by the physician. Ultimately, the quantity of compound administered will be commensurate with the nature of the disease or physiological condition being treated and the therapeutic benefits and the presence or absence of side effects produced by a given dosage regimen, and will be at the discretion of the physician.

Pharmaceutical Formulations

The invention also provides compounds as hereinbefore defined in the form of pharmaceutical compositions.

The pharmaceutical compositions can be in any form suitable for oral, parenteral, topical, intranasal, intrabronchial, ophthalmic, otic, rectal, intra-vaginal, or transdermal administration. Where the compositions are intended for parenteral administration, they can be formulated for intravenous, intramuscular,

intraperitoneal, subcutaneous administration or for direct delivery into a target organ or tissue by injection, infusion or other means of delivery.

Pharmaceutical dosage forms suitable for oral administration include tablets, capsules, caplets, pills, lozenges, syrups, solutions, sprays, powders, granules, elixirs and suspensions, sublingual tablets, sprays, wafers or patches and buccal patches.

Pharmaceutical compositions containing the dihydrotetrabenazine compounds of the invention can be formulated in accordance with known techniques, see for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA.

Thus, tablet compositions can contain a unit dosage of active compound together with an inert diluent or carrier such as a sugar or sugar alcohol, e.g.; lactose, sucrose, sorbitol or mannitol; and/or a non-sugar derived diluent such as sodium carbonate, calcium phosphate, talc, calcium carbonate, or a cellulose or derivative thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose, and starches such as corn starch. Tablets may also contain such standard ingredients as binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures. Such excipients are well known and do not need to be discussed in detail here.

Capsule formulations may be of the hard gelatin or soft gelatin variety and can contain the active component in solid, semi-solid, or liquid form. Gelatin capsules can be formed from animal gelatin or synthetic or plant derived equivalents thereof.

The solid dosage forms (e.g.; tablets, capsules etc.) can be coated or un-coated, but typically have a coating, for example a protective film coating (e.g. a wax or varnish) or a release controlling coating. The coating (e.g. a Eudragit ™ type polymer) can be designed to release the active component at a desired location within the gastro-intestinal tract. Thus, the coating can be selected so as to degrade

under certain pH conditions within the gastrointestinal tract, thereby selectively release the compound in the stomach or in the ileum or duodenum.

Instead of, or in addition to, a coating, the drug can be presented in a solid matrix comprising a release controlling agent, for example a release delaying agent which may be adapted to selectively release the compound under conditions of varying acidity or alkalinity in the gastrointestinal tract. Alternatively, the matrix material or release retarding coating can take the form of an erodible polymer (e.g. a maleic anhydride polymer) which is substantially continuously eroded as the dosage form passes through the gastrointestinal tract.

Compositions for topical use include ointments, creams, sprays, patches, gels, liquid drops and inserts (for example intraocular inserts). Such compositions can be formulated in accordance with known methods.

Compositions for parenteral administration are typically presented as sterile aqueous or oily solutions or fine suspensions, or may be provided in finely divided sterile powder form for making up extemporaneously with sterile water for injection.

Examples of formulations for rectal or intra-vaginal administration include pessaries and suppositories which may be, for example, formed from a shaped mouldable or waxy material containing the active compound.

Compositions for administration by inhalation may take the form of inhalable powder compositions or liquid or powder sprays, and can be administrated in standard form using powder inhaler devices or aerosol dispensing devices. Such devices are well known. For administration by inhalation, the powdered formulations typically comprise the active compound together with an inert solid powdered diluent such as lactose.

The compounds of the inventions will generally be presented in unit dosage form and, as such, will typically contain sufficient compound to provide a desired level of biological activity. For example, a formulation intended for oral administration may contain from 2 milligrams to 200 milligrams of active ingredient, more usually

from 10 milligrams to 100 milligrams, for example, 12.5 milligrams, 25 milligrams and 50 milligrams.

The active compound will be administered to a patient in need thereof (for example a human or animal patient) in an amount sufficient to achieve the desired therapeutic effect.

EXAMPLES

The following non-limiting examples illustrate the synthesis and properties of the dihydrotetrabenazine compounds of the invention.

In the examples, the compounds prepared were characterised by NMR, analytical HPLC and mass spectroscopy (MS, ES^+).

Mass spectra were obtained using a Platform II instrument

NMR spectra were obtained using a Varian Mercury 300 MHz instrument using CD_3OD as solvent.

HPLC analysis was carried out using the following conditions:

Column:	Prodigy ODS(3) 5 µm 100 Å 250 x 4.6 mm		
Mobile phase A:	10 mM ammonium acetate solution (pH 8.0)		
Mobile phase B:	acetonitrile : 10 mM ammonium acetate solution (pH 8.0) (9:1 v/v)		
Gradient	time (min)	A (%)	B (%)
	0.0	90	10
	30.0	30	70
	36.0	10	90
	40.0	10	90
	40.1	90	10
	45.0	90	10
Flow:	1.0 ml min^{-1}		
Temperature:	25°C		
UV:	236 nm		
Inj.:	10 µl, 1 mg ml^{-1} in water : acetonitrile (1:1 v/v)		

EXAMPLE 1

9-Desmethyltetrabenazine

1A. Preparation of 4-Methoxy-3-tosyloxybenzaldehyde

Isovanillin (20.17 g, 0.13 mol) (Aldrich, 07814AC), tosyl chloride (26.24 g, 0.14 mol), and tetrabenzylammonium chloride (80 mg, 0.35 mmol) were dissolved in

dichloromethane (200 ml) with stirring at room temperature. A solution of sodium hydroxide (5.62 g, 0.14 mol) in water (50 ml) was added to the stirred reaction and the organic phase which separated was observed to turn yellow. The reaction mixture was stirred for three days at room temperature. The reaction aqueous layer was separated and the organic layer was washed with 2M aqueous hydrochloric acid (50 ml), dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to give a pale brown solid (40.2 g). This was identified as 4-methoxy-3-tosyloxybenzaldehyde from its ^{1}H-NMR spectrum.

1B. 4-Methoxy-3-tosyloxybenzyl alcohol

4-Methoxy-3-tosyloxybenzaldehyde (10.0 g, 33 mmol) and sodium borohydride (1.37 g, 36 mmol) were stirred together in a mixture of dichloromethane : methanol (10:1) (50 ml) at room temperature. Care was taken to ensure that the ratio of dichloromethane to methanol in the solvent mixture did not exceed 10 to 1, because unwanted side products are formed at higher ratios. The reaction mixture was stirred at room temperature and TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] of an aliquot of the reaction mixture after two hours showed no starting material remained. The reaction mixture was concentrated to dryness at reduced pressure and the residue dissolved in dichloromethane (100 ml). The organic solution was washed with water (100 ml), dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a solid, (10.0 g). This was identified as 4-Methoxy-3-tosyloxybenzyl alcohol from its ^{1}H-NMR spectrum

1C. 4-Methoxy-3-tosyloxybenzyl chloride

In a flask pre-cooled to 0°C, 4-methoxy-3-tosyloxybenzyl alcohol (7.8 g, 25.32 mmol) was treated with thionyl chloride (2.2 ml, 30.39 mmol), which was added dropwise with stirring to form a solution. The reaction mixture was stirred and heated at 60 °C and TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] of an aliquot of the reaction mixture after two hours showed no starting material remained. The reaction mixture was concentrated at reduced pressure and the residue taken up in dichloromethane (100 ml). The organic solution was washed with water (2 x 50 ml), dried over anhydrous potassium carbonate, filtered and

concentrated once more at reduced pressure to a solid residue (7.69 g). This was identified as 4-Methoxy-3-tosyloxybenzyl chloride from its ^{1}H-NMR spectrum.

1D. 4-Methoxy-3-tosyloxyphenylacetonitrile

4-Methoxy-3-tosyloxybenzyl chloride (7.70 g, 23.6 mmol) was dissolved with stirring in dry acetonitrile (250 ml) and 18-crown-6 (7.48 g, 28.3 mmol) (Alfa Aesar, 10127224) and potassium cyanide (1.85 g, 28.3) were added. The reaction mixture was stirred at reflux under an argon atmosphere for twenty four hours and appeared complete following TLC analysis [silica, eluting with ethyl acetate : hexane (1:3)] of an aliquot drawn from the reaction. The reaction mixture was allowed to cool and was then concentrated at reduced pressure. The residue was dissolved in dichloromethane (100 ml) and the solution washed with water (100 ml). The organic layer was separated, dried over potassium carbonate, filtered and concentrated at reduced pressure to give an oil which solidified on standing. This was re-crystallised from methanol to give the product (5.8 g). This was identified as 4-methoxy-3-tosyloxyphenylacetonitrile from its ^{1}H-NMR spectrum.

1E. 4-Methoxy-3-tosyloxyphenylethylamine

4-Methoxy-3-tosyloxyphenylacetonitrile (31 g, 98 mmol) was dissolved with stirring in anhydrous tetrahydrofuran (250 ml) under an argon atmosphere. A solution of borane in THF (800 ml, 1M) was added dropwise at room temperature to the stirring reaction under argon pressure via a cannula. The reaction mixture was then stirred at room temperature overnight before quenching by dropwise addition of methanol (250 ml) to the stirring mixture. The reaction mixture was then concentrated at reduced pressure and the residue taken up in a mixture of methanol (150 ml) and hydrochloric acid (375 ml, approx. 5M). The reaction mixture was allowed to stir for one hour before sodium hydroxide solution (30 %) was added until the pH was >11. The resulting solution was extracted with dichloromethane (2 x 200 ml, 2 x 100 ml) and the combined organic extracts were dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure. The residue was taken up in hydrochloric acid (200 ml, 1 M) and washed with diethyl ether (3 x 100 ml). The aqueous layer was basified as above with sodium hydroxide solution (30 %) until the pH > 11 and was extracted with dichloromethane (2 x 200 ml, 2 x 100

ml). The combined organic extracts were dried over potassium carbonate, filtered and concentrated at reduced pressure to give a solid residue (28.2 g). This was identified as 4-methoxy-3-tosyloxyphenylethylamine from its ^{1}H-NMR spectrum and ^{13}C-NMR spectrum.

1F. N-2-(4-Methoxy-3-hydroxyphenyl)ethyl formamide

Potassium hydroxide (22 g, 392.1 mmol) was dissolved with stirring in ethanol (95 %) : water (1:1) (100 ml), 4-methoxy-3-tosyloxyphenylethylamine (15 g, 46.7 mmol) was added to the stirred solution and the reaction mixture was stirred and heated at 90 °C for one hour. The reaction mixture was then allowed to cool to room temperature and concentrated at reduced pressure to leave a solid residue. The residual solid was then dissolved in methyl formate (70 ml) and the solution stirred at room temperature for two hours during which time a white precipitate formed. TLC analysis [silica, eluting with methanol : dichloromethane (20:80)] of an aliquot drawn from the reaction showed no remaining starting material. The reaction mixture was then concentrated at reduced pressure to leave a solid residue which was partitioned between dichloromethane (100 ml) and water (100 ml). The organic phase was separated and washed with water (2 x 100 ml), dried over anhydrous potassium carbonate, filtered and concentrated once more at reduced pressure to give an oil (6.9 g) which was crystallised from dichloromethane to give the product as a solid (3.5 g). This was identified as N-2-(4-methoxy-3-hydroxyphenyl)ethyl formamide from its ^{1}H-NMR spectrum and ^{13}C-NMR spectrum.

1G. 7-Methoxy-3,4-dihydro-isoquinolin-6-ol

A mixture of N-2-(4-methoxy-3-hydroxyphenyl)ethyl formamide, (6.05 g, 31.0 mmol) and phosphorus oxychloride (3.47 ml, 37.2 mmol) was stirred at reflux in anhydrous acetonitrile (120 ml) for three hours under an argon atmosphere. The reaction was then allowed to cool and was concentrated at reduced pressure to leave an orange oil residue which was suspended in water (20 ml). Ammonium hydroxide solution (30 %, 20 ml) was added slowly to the stirred aqueous suspension which dissolved to form a solution. Silica gel was added to the solution and the mixture was then was concentrated at reduced pressure to until the silica

was free-flowing. The product was purified by column chromatography [silica, eluting with methanol : dichloromethane (10:90)] and the fractions of interest (identified by TLC analysis) were combined and concentrated at reduced pressure to give a yellow solid (3.9 g). This was identified as 7-methoxy-3,4-dihydro-isoquinolin-6-ol from its ^{1}H-NMR spectrum.

1H. 9-Desmethyltetrabenazine

7-Methoxy-3,4-dihydro-isoquinolin-6-ol, (0.46 g, 2.6 mmol) was dissolved with stirring in water (50 ml) together with 3-(N,N-dimethylaminomethyl)-5-methyl-2-hexanone (0.9 g, 2.7 mmol). Sodium hydroxide solution (30 %) was added dropwise to the stirred mixture until the pH of the reaction was ca. 8. The reaction mixture was then stirred at room temperature for four days, occasionally checking and adjusting the pH ca. 8 with sodium hydroxide solution (30 %) if required. The reaction mixture gradually turned a brown colour as the reaction progressed. The reaction mixture was then poured into a stirred saturated solution of sodium carbonate (50 ml) and extracted with dichloromethane (4 x 50 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated to give a yellow oil which was purified by column chromatography [silica, eluting with ethyl acetate : hexane (1:1)] and the fractions of interest (identified by TLC analysis) were combined and concentrated at reduced pressure to give a yellow solid (3.9 g) which was crystallised from ethyl acetate : hexane to give a white solid (0.146 g). This was identified as 9-desmethyltetrabenazine from its ^{1}H-NMR spectrum and ^{13}C-NMR spectrum and the mass spectrum was consistent with the expected structure. HPLC analysis gave a purity of 95.7% (peak area %). HPLC - RT: 29.36 MS: M+1: 304.3

EXAMPLE 2

10-Desmethyltetrabenazine

2A. 3-Methoxy-4-tosyloxybenzaldehyde

A mixture of vanillin (101 g, 0.66 mol) (Aldrich, 026K3740), potassium carbonate (96 g, 0.69 mol) and p-toluenesulphonyl chloride (134 g, 0.69 mol) was dissolved with stirring in acetone (2 L) at room temperature under argon. A white suspension formed in the pink reaction solution and the reaction mixture was stirred overnight

at room temperature. The reaction mixture consisted of a colourless solution and a white suspension and the acetone solvent was removed at reduced pressure to give a solid residue. The solid residue was taken up in dichloromethane (800 ml) with stirring and water (300 ml) was added to the mixture. The aqueous phase was removed and the organic solution was washed further with water (3 x 300 ml), dried over anhydrous magnesium sulphate, filtered and concentrated in vacuo to give a pale yellow solid (197.8 g). TLC analysis [silica, eluting with dichloromethane] showed no starting material remained and a single product component which was identified as 3-methoxy-4-tosyloxybenzaldehyde from its ^{1}H-NMR spectrum and ^{13}C-NMR spectrum.

2B. 3-Methoxy-4-tosyloxybenzyl alcohol

3-Methoxy-4-tosyloxybenzaldehyde (197.6 g, 0.65 mol) was dissolved with stirring in methanol (750 ml) and dichloromethane (750 ml) at room temperature. Sodium borohydride (49.6 g, 1.31 mol) was added in portions to the stirred solution and the reaction mixture was stirred for a further 150 min. at room temperature. TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] of an aliquot from the reaction mixture (partitioned between water and ethyl acetate, organic layer analysed) and analysing the ethyl acetate layer showed no starting material remained. The reaction solvent was evaporated at reduced pressure and the residual slurry was taken up in dichloromethane (800 ml) and water (300 ml). The two-phase mixture was poured into a separating funnel and the aqueous layer was removed. The organic layer was washed further with water (2 x 150 ml), dried over anhydrous magnesium sulphate, filtered and concentrated at reduced pressure to give a pale brown solid (178.2 g). TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] showed no starting material and a single product component which was identified as 3-methoxy-4-tosyloxybenzyl alcohol from its ^{1}H-NMR spectrum.

2C. 3-Methoxy-4-tosyloxybenzyl chloride

3-Methoxy-4-tosyloxybenzyl alcohol (170 g, 0.55 mol) was cooled to 0°C under argon using an ice bath and thionyl chloride (50 ml, 0.69 mol) was added slowly with stirring to form a solution. The reaction mixture was stirred and heated under argon at 50°C for five hours to give a dark green solution. TLC analysis [silica,

eluting with dichloromethane : hexane (3:1)] of an aliquot from the reaction mixture showed no starting material remained. The reaction solvent was removed at reduced pressure and the residual syrup taken up in dichloromethane (800 ml) before water (300 ml) was added. The two-phase reaction mixture was poured into a separating funnel and the aqueous layer was removed. The organic layer was washed further with water (2 x 150 ml), dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a dark green syrup which was crystallised from hot methanol to give a pale yellow solid (129.0 g). TLC analysis [silica, eluting with dichloromethane : hexane (3:1)] showed no remaining starting material and a single product component which was identified as 3-methoxy-4-tosyloxybenzyl chloride from its ^{1}H-NMR spectrum.

2D. 3-Methoxy-4-tosyloxyphenylacetonitrile

A mixture of 3-methoxy-4-tosyloxybenzyl chloride (56 g, 0.17 mol), 18-crown-6 (55 g, 0.21 mol) and potassium cyanide (14 g, 0.21 mol) was dissolved with stirring in dry acetonitrile (500 ml) under argon to give a green solution with a white suspension. The reaction mixture was then stirred at reflux for six hours and allowed to cool before the solvent was removed at reduced pressure. The residual syrup was taken up in dichloromethane (1 L) and water (300 ml) was added before the mixture was poured into a separating funnel and the aqueous layer removed. The organic layer was washed further with water (2 x 250 ml), dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a purple syrup that crystallised on standing at room temperature overnight. The crude product was crystallised twice using hot methanol to give a pale pink solid (37.4 g). TLC analysis [silica, eluting with dichloromethane] showed no starting material remained and a single product component which was identified as 3-methoxy-4-tosyloxyphenylacetonitrile from its ^{1}H-NMR spectrum.

2E. N-2-(3-Methoxy-4-hydroxyphenyl)ethyl formamide

3-Methoxy-4-tosyloxyphenylacetonitrile (35.3 g, 0.11 mol) was dissolved with stirring in dry tetrahydrofuran (250 ml) under argon at room temperature over twenty minutes. A solution of 1M borane in THF (800 ml, 0.80 mol) was added dropwise via cannula to the stirred reaction mixture over forty five minutes to give

a clear yellow solution which was then was stirred at room temperature overnight. TLC analysis [silica, eluting with methanol : dichloromethane (2:8)] of an aliquot from the reaction mixture showed no starting material remained. The stirred reaction mixture was cooled to 0 °C and methanol (150 ml) was slowly added dropwise (caution: a large amount of hydrogen gas is evolved) and once the initial vigorous reaction had subsided an additional quantity of methanol (350 ml) was added to ensure no borane remained. The solvent was removed at reduced pressure and the residual foam dissolved using 5 M hydrochloric acid (500 ml) and methanol (1 L). The resulting solution was stirred at room temperature for one hour before it was basified by addition of aqueous sodium hydroxide (ca. 2 M). A white precipitate was formed and the reaction mixture was poured into a separating funnel and extracted with dichloromethane (10 x 100 ml). The organic extracts were combined, dried over anhydrous potassium carbonate and concentrated at reduced pressure to give a brown viscous oil (33.4 g). The oil was dissolved in 1 M hydrochloric acid (500 ml), and the solution poured into a separating funnel and washed with diethyl ether (2 x 200 ml). The acidic aqueous layer was made basic by the addition of 1M sodium hydroxide and then extracted with dichloromethane (10 x 200 ml). The organic extracts were combined, dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give the amine intermediate as an orange oil which was used without further purification. The orange oil (23.4 g) was dissolved with stirring in ethanol (300 ml) and potassium hydroxide (22.0 g, 392.9 mmol) in water (300 ml) was added to the reaction mixture. A cloudy solution was formed which was stirred and heated to 100°C for one hour during which time the reaction solution became clear. TLC analysis [silica, eluting with methanol : dichloromethane (2:8)] of an aliquot from the reaction mixture showed no starting material remained and the solvents were therefore removed at reduced pressure to give a yellow solid residue. The yellow solid was dissolved with stirring in methyl formate (500 ml) and the solution was stirred at room temperature for two days until TLC analysis [silica, eluting with methanol : dichloromethane (10:90)] of an aliquot from the reaction mixture showed no starting material remained. The reaction mixture was filtered and the filtrate concentrated at reduced pressure leaving a residual syrup which was dissolved in dichloromethane (500 ml) and water (200 ml). The mixture was

transferred to a separating funnel and the aqueous layer was removed. The organic layer was washed further with water (2 x 200 ml), dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give crude an orange syrup (12.0 g). The orange syrup was purified by column chromatography [silica, eluting with methanol : dichloromethane (4:96)] to give a honey coloured syrup (7.4 g). TLC analysis [silica, eluting with methanol : dichloromethane (10:90)] showed no remaining starting material and a single product component which was identified as N-2-(3-methoxy-4-hydroxyphenyl)ethyl formamide from its ^{1}H-NMR spectrum.

2F. 6-methoxy-3,4-dihydro-isoquinolin-7-ol

N-2-(3-Methoxy-4-hydroxyphenyl)ethyl formamide (7.4 g, 38.0 mmol) was dissolved with stirring in acetonitrile (150 ml) with gentle heating under an argon atmosphere. Phosphorous oxychloride (4.3 ml, 45.6 mmol) was added to the solution and the honey coloured reaction mixture was stirred at reflux for four hours during which time a clear dark red solution formed. TLC analysis of an aliquot from the reaction [silica, eluting with methanol : dichloromethane (10:90)] showed no starting material remained. The solvent was removed at reduced pressure and the residue was taken up in 1M hydrochloric acid (500 ml) – sonication for 10 minutes gave an orange solution and a brown precipitate. The acidic mixture was basified by addition of ammonium hydroxide (30 %) and then extracted with dichloromethane (10 x 200 ml). The organic extracts were combined, dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a yellow solid (4.3 g) which was purified by crystallisation in hot methanol to give a yellow crystalline solid (3.2 g). TLC analysis [silica, eluting with methanol : dichloromethane (10:90)] showed a single product component which was identified as 6-methoxy-3,4-dihydro-isoquinolin-7-ol from its ^{1}H-NMR spectrum.

2G. 10-Desmethyltetrabenazine

6-Methoxy-3,4-dihydro-isoquinolin-7-ol, (2.00 g, 9.95 mmol) was dissolved with stirring in water (200 ml) together with 3-(N,N-dimethylaminomethyl)-5-methyl-2-hexanone (1.87 g, 10.95 mmol). Aqueous sodium hydroxide (30 %) was added dropwise to adjust the reaction to pH 8 and the reaction was then stirred at room

temperature for four days. During this time the reaction pH was monitored and adjusted to pH 8 by the dropwise addition of aqueous sodium hydroxide (30 %) solution if required. The reaction mixture gradually turned brown in colour. The reaction mixture was then poured into a saturated aqueous solution of sodium carbonate (200 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated to give a yellow oil which was purified using column chromatography [silica, eluting with ethyl acetate : hexane (1:1)]. The fractions of interest were identified by TLC analysis and combined and concentrated at reduced pressure to give a yellow solid residue which was crystallised from ethyl acetate : hexane give a white solid (0.278 g). This was identified as 10-desmethyl-tetrabenazine from its ^{1}H-NMR spectrum and ^{13}C-NMR spectrum and the mass spectrum was consistent with the expected structure. HPLC analysis gave a purity of 97.9% (peak area %). HPLC - RT: 29.5 MS: M+1: 304.3

EXAMPLE 3

9-Desmethyl-α-dihydrotetrabenazine

A mixture of 9-desmethyltetrabenazine (1.00 g, 3.3 mmol) and sodium borohydride (0.25 g, 6.6 mmol) was stirred in a mixture of methanol : dichloromethane (1:1) (100 ml) at room temperature. TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] of an aliquot of the reaction mixture after one hour showed no starting material remained. The reaction mixture was then concentrated to dryness at reduced pressure and the residual solid partitioned between water (50 ml) and dichloromethane (50 ml). The organic layer was separated and the aqueous layer further extracted with dichloromethane (2 x 50 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a solid which was crystallised from methanol to give a white solid (0.27 g). The ^{1}H-NMR spectrum and the mass spectrum were consistent with the structure of 9-desmethyl-α-dihydrotetrabenazine. HPLC analysis gave a purity of 97.3% (peak area %). HPLC - RT: 22.11 MS: M+1: 306.2

EXAMPLE 4

9-Desmethyl-β-dihydrotetrabenazine

9-Desmethyltetrabenazine (1.30 g, 4.28 mmol) was dissolved with stirring at 0°C (ice bath) in dry tetrahydrofuran (30 ml) under an argon atmosphere. A solution of L-selectride (8.50 ml, 1M in THF) was added dropwise at 0 °C via a syringe to the stirring solution and the reaction mixture was then allowed to warm to room temperature. The reaction mixture was stirred for three hours and was then poured into water (40 ml) containing glacial acetic acid (4 ml). The resulting mixture was then washed with diethyl ether (2 x 30 ml) and was made alkaline (pH = 9) by addition of solid sodium carbonate. This alkaline mixture was then extracted using dichloromethane (3 x 60 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a solid residue which was crystallised from methanol : ethyl acetate to give a white solid (0.44 g). The ^{1}H-NMR spectrum and the mass spectrum were consistent with the structure of 9-desmethyl-β-dihydrotetrabenazine. HPLC analysis gave a purity of 98.2% (peak area %). HPLC - RT: 19.01 MS: M+1: 306.2

EXAMPLE 5

10-Desmethyl-α-dihydrotetrabenazine

10-Desmethyltetrabenazine (1.30 g, 4.3 mmol) and sodium borohydride (0.32 g, 8.6 mmol) were stirred together in a mixture of methanol : dichloromethane (1:1) (100 ml) at room temperature. TLC analysis [silica, eluting with ethyl acetate : hexane (1:1)] of an aliquot of the reaction mixture after one hour showed no starting material remained. The reaction mixture was then concentrated to dryness at reduced pressure and the residual solid partitioned between water (50 ml) and dichloromethane (50 ml). The organic layer was separated and the aqueous layer further extracted with dichloromethane (2 x 50 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a solid which was crystallised from methanol to give a white solid (0.477 g). The ^{1}H-NMR spectrum and the mass spectrum were consistent with the structure of 10-Desmethyl-α-dihydrotetrabenazine. HPLC analysis gave a purity of 98.6 % (peak area %). HPLC - RT: 21.28 MS: M+1: 306.3

EXAMPLE 6

10-Desmethyl-β-dihydrotetrabenazine

10-Desmethyltetrabenazine (1.50 g, 4.9 mmol) was dissolved with stirring at 0°C (ice bath) in dry tetrahydrofuran (30 ml) under an argon atmosphere. A solution of L-selectride (10.00 ml, 1M in THF) was added dropwise at 0°C via syringe to the stirring solution and the reaction mixture was then allowed to warm to room temperature. The reaction mixture was stirred for three hours and was then poured into water (40 ml) containing glacial acetic acid (4 ml). The resulting mixture was washed with diethyl ether (2 x 30 ml) and was made alkaline (pH = 9) by the addition of solid sodium carbonate. This alkaline mixture was then extracted using dichloromethane (3 x 60 ml). The combined organic layers were dried over anhydrous potassium carbonate, filtered and concentrated at reduced pressure to give a solid residue which was crystallised from methanol : ethyl acetate to give a white solid (0.91 g). The ^{1}H-NMR spectrum and the mass spectrum were consistent with the structure of 10-Desmethyl-β-dihydrotetrabenazine. HPLC analysis gave a purity of 98.3 % (peak area %). HPLC - RT: 19.84 MS: M+1: 306.3

BIOLOGICAL ACTIVITY

EXAMPLE 7

Screen for VMAT-2 binding activity using a [^{3}H] Dihydrotetrabenazine binding assay

Dihydrotetrabenazine is a very potent and selective inhibitor of VMAT-2, and binds with high affinity (nM range) to this vesicular transporter. [^{3}H] Dihydro-tetrabenazine has been successfully used for many years as a radioligand to label VMAT-2 in human, bovine and rodent brain (e.g. Scherman *et al. J. Neurochem.* 50, 1131-1136 (1988); Near *et al. Mol. Pharmacol.* 30, 252-257 (1986); Kilbourn *et al. Eur. J. Pharmacol.* 278, 249-252 (1995); and Zucker *et al. Life Sci.* 69, 2311-2317 (2001)).

The compounds of the invention can be tested for their ability to inhibit the VMAT-2 transporter using the assay described below.

Methods and Materials

Adult rat (Wistar strain) forebrain membranes are prepared essentially as described by Chazot *et al.* (1993) *Biochem. Pharmacol.* 45, 605-610. Adult rat striatal vesicular membranes are prepared essentially as described by Roland *et al.* (2000) *JPET* 293, 329-335. 10 µg Membranes are incubated at 25°C with [^{3}H] dihydrotetrabenazine (18-20 nM) in 50mM HEPES pH 8.0 (assay buffer), for 60 minutes, and bound radioligand is collected by rapid filtration under vacuum on GF/B glass-fibre filters. Non-specific binding is determined in parallel samples in the presence of 2 µM unlabelled tetrabenazine. Radioactivity is counted in scintillation fluid in a β-counter. A full concentration range (log and half-log units) of the test compounds is assayed (range: 10^{-11} - 10^{-4}M) in triplicate. Test compounds and tetrabenazine are dissolved in DMSO at a stock concentration of 10 mM, and dilutions then prepared in assay buffer. Three independent experiments are performed for each compound. Data are analysed and curve fitted using the GraphPad Prism 3.2 package.

EXAMPLE 8

VMAT Functional Assays

A. VMAT2 Functional Assay

Rat striatal synaptic vesicles are prepared essentially as described in Example 7. Thus, a rat striatal P_2 membrane preparation (Chazot *et al.*, 1993) is resuspended and homogenised in ice-cold distilled water. Osmolarity is restored by addition of 25 mM HEPES and 100 mM potassium tartrate (pH 7.5, 4C). The preparation is then centrifuged for 20 minutes at 20,000 x g (4 °C). The resultant S_2 fraction is removed, magnesium sulphate is added (to give a final concentration of 1 mM, pH 7.5, 4 °C), and the mixture is centrifuged at 100,000 x g for 45 minutes. The final P_4 fraction contains the synaptic vesicles for the assay.

An aliquot of 100 µl (approx. 2.5 µg protein) of synaptic vesicles is preincubated with increasing concentrations of the test compounds (prepared fresh as a stock of 10^{-4}M in DMSO) for 30 minutes (concentration range 10^{-9} M – 10^{-4} M), and then for 3 minutes in the assay buffer (25 mM HEPES, 100 mM potassium tartrate, 1.7 mM ascorbic acid, 0.05 mM EGTA, 0.1 mM EDTA, 2 mM ATP-Mg^{2+}, pH 7.5), in

the presence of [^{3}H] dopamine (30 nM final concentration) at 30 °C. The reaction is then terminated by addition of ice-cold buffer assay buffer pH 7.5, containing 2 mM $MgSO_4$ instead of 2 mM ATP-Mg^{2+}, and rapid filtration achieved through Whatman filters soaked in 0.5% polyethyleneimine. The filters are washed three times with cold buffer using a Brandel Harvester. The radioactivity trapped on the filters is counted using a liquid scintillation counter and non-specific binding is determined by measuring vesicular [^{3}H] dopamine uptake at 4 °C. The method is based on that described in Ugarte YV *et al.* (2003) *Eur. J. Pharmacol.* **472**, 165-171. Selective VMAT-2 uptake is defined using 10 µM tetrabenazine.

B. VMAT1 functional assay

There are very limited native tissues which possess VMAT1 alone, in isolation from VMAT2. However, tetrabenazine displays at least a 200-fold higher affinity for VMAT2 in comparison to VMAT1, and this discrimination can be used to block the influence of VMAT2 in the functional assay (Erickson *et al.* (1996) *PNAS (USA)* **93**, 5166-5171). Adrenal chromaffin cells are isolated from young adult SD rats essentially as described in Moshharov *et al.* (2003) *J Neurosci.* **23**, 5835-5845. Thus, adrenal glands are dissected in ice cold PBS, the capsule and cortex of the glands removed and the remaining medullae are minced. After multiple washes with PBS, the tissue is incubated with Ca2+-free collagenase IA solution (250U/ml) for 30 minutes at 30 °C with gentle stirring. The digested tissue is rinsed three times and the dissociated cells are centrifuged at 3000 rpm to form a pellet, which is resuspended in PBS. The vesicular fraction is isolated in an identical fashion to that described for the brain preparation.

100 µl (approx. 2.5 µg protein) of synaptic vesicles are preincubated with increasing concentrations of test compound (prepared as previously described for binding assay) for 30 minutes (concentration range 10^{-9} M – 10^{-4} M). The assay is performed for 3 minutes at 30 °C in the assay buffer (25 mM HEPES, 100 mM potassium tartrate, 1.7 mM ascorbic acid, 0.05 mM EGTA, 0.1 mM EDTA, 2 mM ATP-Mg^{2+}, pH 7.5), in the presence of [^{3}H] dopamine (30 nM final concentration). [^{3}H] dopamine uptake is measured in the presence of 10 µM tetrabenazine (selectively blocks VMAT2 at this concentration). Non-specific uptake is

determined by measuring vesicular [^{3}H] dopamine uptake at 4 °C. The reaction is then terminated by addition of ice-cold buffer assay buffer pH 7.5, containing 2 mM $MgSO_4$ instead of 2 mM ATP-Mg^{2+}, and rapid filtration achieved through Whatman filters soaked in 0.5% polyethyleneimine. The filters are washed three times with cold buffer using a Brandel Harvester and the radioactivity trapped on the filters is counted using a liquid scintillation counter.

EXAMPLE 9

Receptor and Transporter Protein Binding Studies

Compounds of the invention can be subjected to specific binding assays to test their ability to bind to the receptors and transporter proteins described below.

(a) Adrenergic α_{2A} Receptor:

Reference: S. Uhlçn *et al. J. Pharmacol. Exp. Ther.*, 271:1558-1565 (1994)
Source: Human recombinant insect Sf9 cells
Ligand: 1 nM [^{3}H] MK-912
Vehicle: 1% DMSO
Incubation time/Temp: 60 minutes @ 25 °C
Incubation buffer: 75mM Tris-HCl, pH 7.4, 12.5mM $MgCl_2$, 2mM EDTA
Non Specific ligand: 10μM WB-4101
K_d: 0.6 nM
B_{max}: 4.6 pmole/mg protein
Specific binding: 95%
Quantitation method: Radioligand binding
Significance criteria: ≥ 50% of maximum stimulation or inhibition

(b) Adrenergic α_{2B} Receptor:

Reference: S. Uhlen *et al., Eur. J. Pharmacol.*, 33 (1): 93-1-1 (1998)
Source: Human recombinant CHO-K1 cells
Ligand: 2.5 nM [3H] Rauwolscine
Vehicle: 1% DMSO
Incubation time/Temp: 60 minutes @ 25 °C

Incubation buffer: 50 mM Tris-HCl, 1 mM EDTA, 12.5mM $MgCl_2$, pH 7.4, 0.2% BSA at 25 °C

Non Specific ligand: 10 μM Prazosin

K_d: 2.1 nM

B_{max}: 2.1 pmole/mg protein

Specific binding: 90%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(c) Dopamine D_3 Receptor:

Reference: Dearry *et al.*, *Nature*, 347:72-76, (1990)

Source: Human recombinant CHO cells

Ligand: 1.4 nM [3H] SCH-23390

Vehicle: 1% DMSO

Incubation time/Temp: 2 hours @ 37 °C

Incubation buffer: 50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA

Non Specific ligand: 10 μM (+)-butaclamol

K_d: 1.4 nM

B_{max}: 0.63 pmole/mg protein

Specific binding: 90%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(d) Dopamine D_{2L} Receptor:

Reference: Bunzo *et al.*, *Nature*, 336:783-787 (1988)

Source: Human recombinant CHO cells

Ligand: 0.16 nM [3H] Spiperone

Vehicle: 1% DMSO

Incubation time/Temp: 2 hours @ 25 °C

Incubation buffer: 50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA

Non Specific ligand: 10 μM Haloperidol

K_d: 0.08 nM

B_{max}: 0.48 pmole/mg protein

Specific binding: 85%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(e) Dopamine D_3 Receptor:

Reference: Sokoloff *et al.*, *Nature*, 347:146-151, (1990)

Source: Human recombinant CHO cells

Ligand: 0.7 nM [3H] Spiperone

Vehicle: 1% DMSO

Incubation time/Temp: 2 hours @ 37 °C

Incubation buffer: 50 mM Tris-HCl, pH 7.4, 150 nM NaCl, 1.4 nM ascorbic acid, 0.001% BSA

Non Specific ligand: 25 μM S(-)-Sulpiride

K_d: 0.36 nM

B_{max}: 1.1 pmole/mg protein

Specific binding: 85%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(f) Imidazoline I_2 (Central) Receptor:

Reference: Brown *et al.*, *Brit. J. Pharmacol.*, 99:803-809, (1990)

Source: Wistar rat cerebral cortex

Ligand: 2 nM [3H] Idazoxan

Vehicle: 1% DMSO

Incubation time/Temp: 30 minutes @ 25°C

Incubation buffer: 50 mM Tris-HCl, 0.5 mM EDTA, pH 7.4 at 25 °C

Non Specific ligand: 1 μM Idazoxan

K_d: 4 nM

B_{max}: 0.14 pmole/mg protein

Specific binding: 85%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(g) Sigma σ_1 Receptor:

Reference: Ganapathy *et al.*, *Pharmacol. Exp. Ther.*, 289:251-260, (1999)

Source: Human jurkat cells

Ligand: 8 nM [3H] Haloperidol

Vehicle: 1% DMSO

Incubation time/Temp: 4 hours @ 25 °C

Incubation buffer: 5 mM K2HPO4/KH2PO4 buffer pH 7.5

Non Specific ligand: 10 μM Haloperidol

K_d: 5.8 nM

B_{max}: 0.71 pmole/mg protein

Specific binding: 80%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(h) Sigma σ_2 Receptor:

Reference: Hashimoto *et al.*, *Eur. J. Pharmacol.*, 236:159-163, (1993)

Source: Wistar rat brain

Ligand: 3 nM [3H] Ifenprodil

Vehicle: 1% DMSO

Incubation time/Temp: 60 minutes @ 37 °C

Incubation buffer: 50 mM Tris-HCl, pH 7.4

Non Specific ligand: 10 μM Ifenprodil

K_d: 4.8 nM

B_{max}: 1.3 pmole/mg protein

Specific binding: 85%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(i) Serotonin Transporter (SERT):

Reference: Gu *et al.*, *J. Biol. Chem.*, 269(10):7124-7130, (1994)

Source: Human recombinant HEK-293 cells

Ligand: 0.15 nM [125I] RTI-55

Vehicle: 1% DMSO

Incubation time/Temp: 3 hours @ 4 °C

Incubation buffer: 100 mM NaCl, 50 mM Tris HCl, 1 μM Leupeptin, 10 μM PMSF, pH 7.4

Non Specific ligand: 10 μM Imipramine

K_d: 0.17 nM

B_{max}: 0.41 pmole/mg protein

Specific binding: 95%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(j) Dopamine Transporter (DAT):

Reference: Giros *et al.*, *Trends Pharmacol. Sci.*, 14, 43-49 (1993)
Gu *et al.*, *J. Biol. Chem.*, 269(10):7124-7130 (1994)

Source: Human recombinant CHO cells

Ligand: 0.15 nM [^{125}I] RTI-55

Vehicle: 1% DMSO

Incubation time/Temp: 3 hours @ 4 °C

Incubation buffer: 100 mM NaCl, 50 mM Tris HCl, 1 μM Leupeptin, 10 μM PMSF, pH 7.4

Non Specific ligand: 10 μM Nomifensine

K_d: 0.58 nM

B_{max}: 0.047 pmole/mg protein

Specific binding: 90%

Quantitation method: Radioligand binding

Significance criteria: ≥ 50% of maximum stimulation or inhibition

EXAMPLE 10

Enzyme Assays

Compounds of the invention can be tested for their ability to inhibit enzymes involved in the processing of monoamines in the CNS, namely Catechol O-Methyl Transferase (COMT), Monoamine Oxidase A and Monoamine Oxidase B.

(a) Catechol O-Methyl Transferase (COMT) Inhibition

Source: Porcine liver

Substrate: 3 mM catechol + S-adenosyl-L-[^{3}H]methionine

Vehicle: 1% DMSO

Pre-incubation time/Temp: None

Incubation time: 60 minutes @ 37 °C

Incubation buffer: 100 mM potassium phosphate, 10mM $MgCl_2$, 3 mM DTT containing 12 units/ml adenosine deaminase, pH 7.4

Quantitation method: Quantitation of [^{3}H] guiacol.

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(b) Monoamine Oxidase MAO-A Inhibition

Source: Human recombinant

Substrate: 50 µM kynuramine

Vehicle: 1% DMSO

Pre-incubation time/Temp: 15 minutes @ 37 °C

Incubation time: 60 minutes @ 37 °C

Incubation buffer: 100 mM KH_2PO_4, pH 7.4

Quantitation method: Spectrofluorimetric quantitation of 4-hydroxyquinoline

Significance criteria: ≥ 50% of maximum stimulation or inhibition

(c) Monoamine Oxidase MAO-B Inhibition

Source: Human recombinant

Substrate: 50 µM kynuramine

Vehicle: 1% DMSO

Pre-incubation time/Temp: 15 minutes @ 37 °C

Incubation time: 60 minutes @ 37 °C

Incubation buffer: 100 mM KH_2PO_4, pH 7.4

Quantitation method: Spectrofluorimetric quantitation of 4-hydroxyquinoline

Significance criteria: ≥ 50% of maximum stimulation or inhibition

EXAMPLE 11

Cellular Assays

The ability of compounds of the invention to inhibit uptake of serotonin (5-hydroxytryptamine) by human embryonic kidney cells can be measured using the following assay conditions:

Target:	Human HEK-293 cells
Vehicle:	0.4 % DMSO
Incubation Time/Temp:	10 minutes @ 25 °C
Incubation buffer:	5mM Tris-HCl, 7.5 mM HEPES, 120 mM NaCl, 5.4 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 5 mM glucose, 1 mM ascorbic acid, pH 7.1
Quantitation Method:	Quantitation of [^{3}H] serotonin uptake
Significance criteria:	≥ 50% inhibition of [^{3}H] serotonin uptake relative to fluxetine response.

EXAMPLE 12

5-HT $_{1D/1B}$ Binding Assay

The ability of the compounds of the invention to bind to 5-HT $_{1D/1B}$ receptors can be tested using an assay based on the one described by Millan, MJ *et al.* (2002) *Pharmacol. Biochem. Behav.* 71, 589-598. [N-methyl ^{3}H] GR-125743 is used as the radioliogand for both 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors. Adult SD rat forebrain P_2 membranes (Chazot *et al.*, 1993) are used for the assay. The assay buffer used is 50 mM Tris-HCl pH 7.7 at room temperature containing 4 mM calcium chloride, 0.1% ascorbic acid and 10 µM pargyline. 5-HT (10 µM) is used to define non-specific binding. Incubation with 1 nM [^{3}H] GR-125743 is carried out for 1 hour at room temperature, and the reaction is terminated by rapid filtration using a Brandel Harvester through GF/B filters pre-soaked in 0.1% polyethyleneimine, followed by three washes with ice-cold buffer (supplemented with 0.1% BSA). A dose range of 10^{-10}-10^{-4}M is utilised. The resultant competition curves are analysed using the GraphPad Prism 4 package.

EXAMPLE 13

Pharmaceutical Compositions

(i) Tablet Formulation -I

A tablet composition containing a compound of the invention is prepared by mixing 50mg of the dihydrotetrabenazine with 197mg of lactose (BP) as diluent, and 3mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.

(ii) Tablet Formulation - II

A tablet composition containing a compound of the invention is prepared by mixing the compound (25 mg) with iron oxide, lactose, magnesium stearate, starch maize white and talc, and compressing to form a tablet in known manner.

(iii) Capsule Formulation

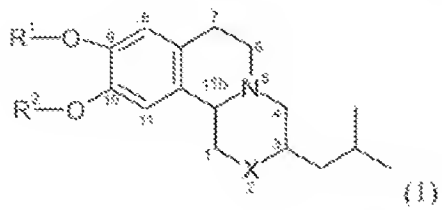
A capsule formulation is prepared by mixing 100mg of a compound of the invention with 100mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

Equivalents

It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the invention described above without departing from the principles underlying the invention. All such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. A pharmaceutical composition comprising a compound of the formula (1):



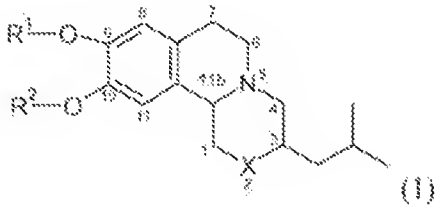
(1)

or a pharmaceutically acceptable salt or tautomer thereof;

wherein R^1 and R^2 are each selected from hydrogen and methyl, provided that at least one of R^1 and R^2 is hydrogen; and X is CHOH or C=O;

and a pharmaceutically acceptable carrier.

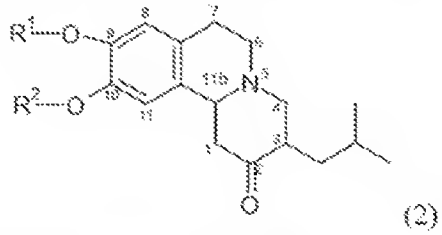
2. A compound of the formula (1):



(1)

or a pharmaceutically acceptable salt or tautomer thereof, as hereinbefore defined but excluding the compounds 2*R*, 3*R*, 11b*R* - 9-O-desmethyl-dihydrotetrabenazine and 2*S*, 3*S*, 11b*S* - 9-O-desmethyl-dihydrotetrabenazine and their salts and tautomers.

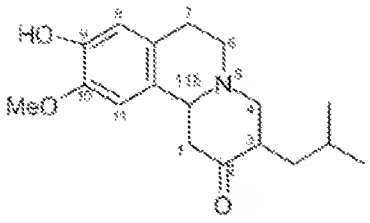
3. A compound according to claim 2 having the formula (2):



(2)

and pharmaceutically acceptable salts thereof.

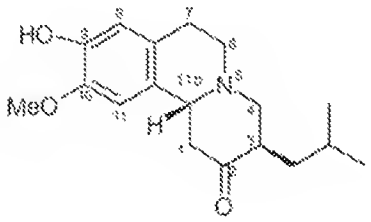
4. A compound according to claim 3 having the formula (2a):



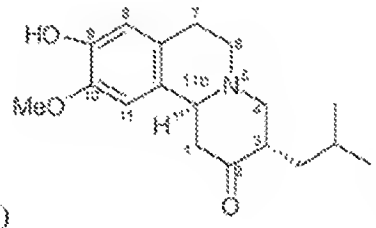
(2a)

and pharmaceutically acceptable salts thereof.

5. A compound according to claim 4 which is selected from the compounds (2a-I) and (2a-II):



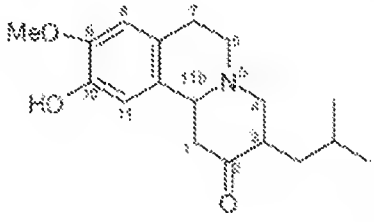
(2a-I)



(2a-II)

and pharmaceutically acceptable salts thereof.

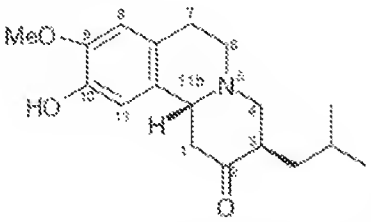
6. A compound according to claim 3 having the formula (2b):



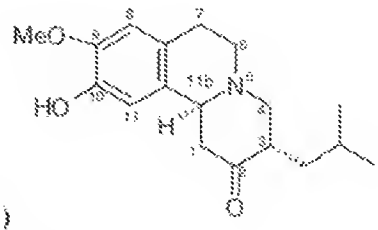
(2b)

and pharmaceutically acceptable salts thereof.

7. A compound according to claim 6 which is selected from compounds (2b-I) and (2b-II):



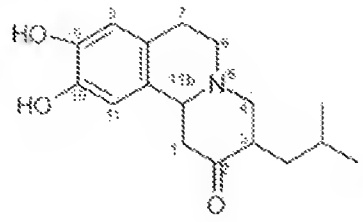
(2b-I)



(2b-II)

and pharmaceutically acceptable salts thereof.

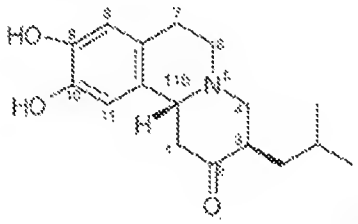
8. A compound according to claim 3 having the formula (2c):



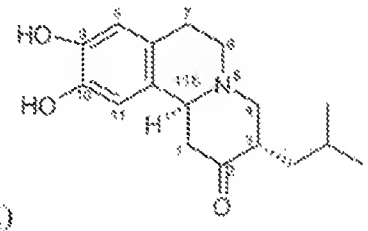
(2c)

and pharmaceutically acceptable salts thereof.

9. A compound according to claim 8 which is selected from the compounds (2c-I) and (2c-II):



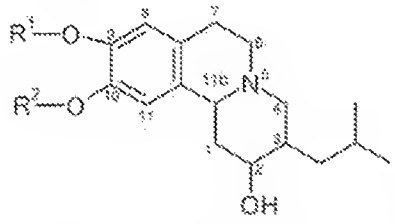
(2c-I)



(2c-II)

and pharmaceutically acceptable salts thereof.

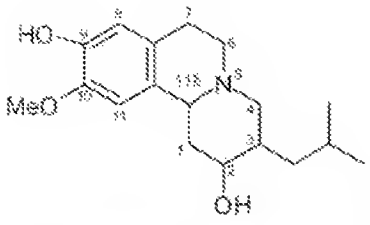
10. A compound according to claim 2 having the formula (3):



(3)

and pharmaceutically acceptable salts thereof, but excluding the compounds 2*R*, 3*R*, 11b*R* - 9-O-desmethyl-dihydrotetrabenazine and 2*S*, 3*S*, 11b*S* - 9-O-desmethyl-dihydrotetrabenazine and their salts and tautomers.

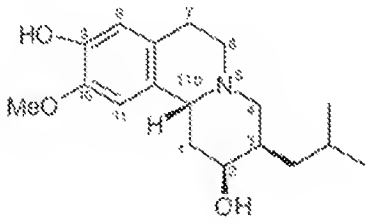
11. A compound according to claim 10 having the formula (3a):



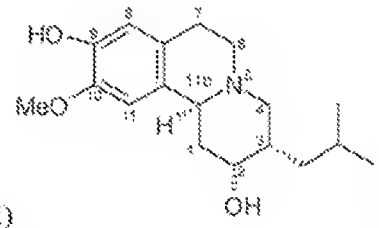
(3a)

and pharmaceutically acceptable salts thereof.

12. A compound according to claim 11 which is selected from the compounds (3a-I) and (3a-II):



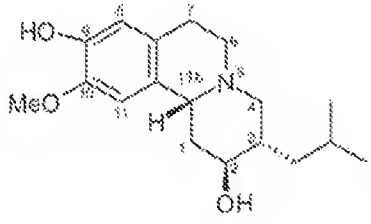
(3a-I)



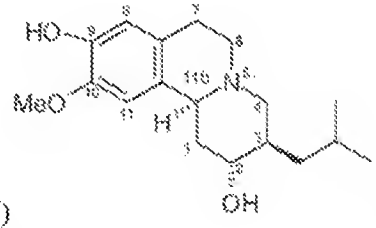
(3a-II)

and salts and tautomers thereof.

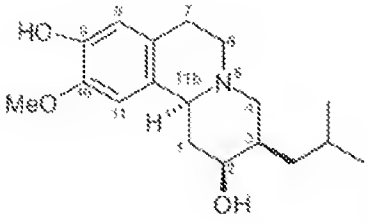
13. A compound according to claim 11 which is selected from the compounds (3a-V), (3a-VI), (3a-VII) and (3a-VIII):



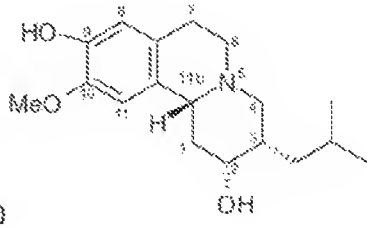
(3a-V)



(3a-VI)



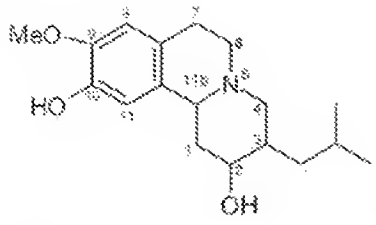
(3a-VII)



(3a-VIII)

and pharmaceutically acceptable salts thereof.

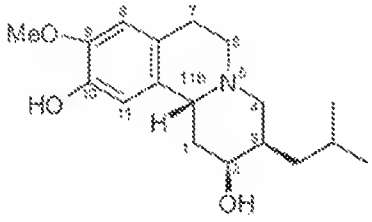
14. A compound according to claim 10 having the formula (3b):



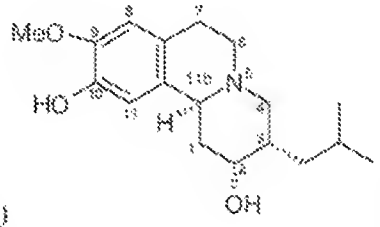
(3b)

and pharmaceutically acceptable salts thereof.

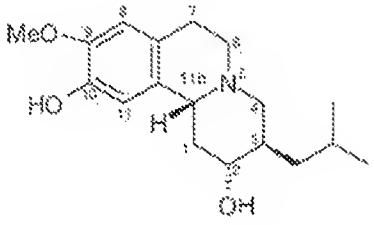
15. A compound according to claim 14 which is selected from the compounds (3b-I), (3b-II), (3b-III) and (3b-IV):



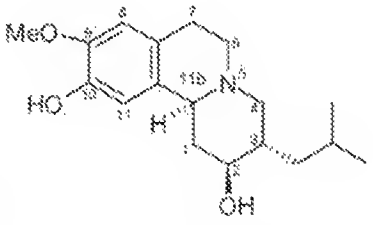
(3b-I)



(3b-II)



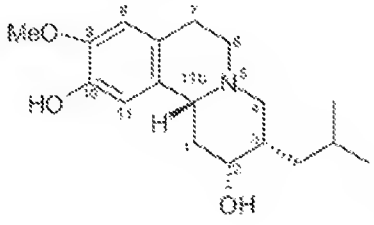
(3b-III)



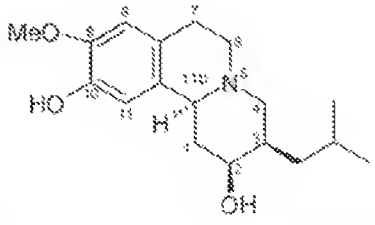
(3b-IV)

and pharmaceutically acceptable salts thereof.

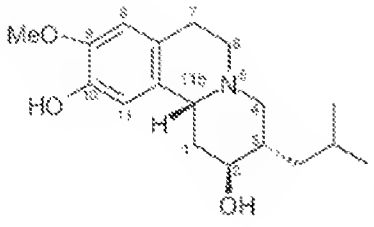
16. A compound according to claim 14 which is selected from the compounds (3b-V), (3b-VI) (3b-VII) and (3b-VIII):



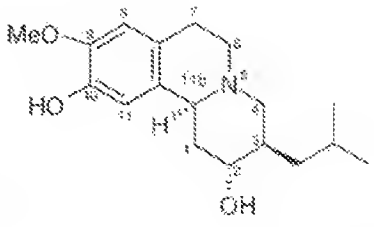
(3b-V)



(3b-VI)



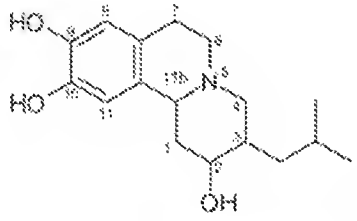
(3b-VII)



(3b-VIII)

and pharmaceutically acceptable salts thereof.

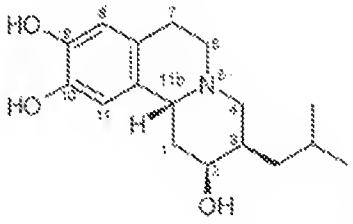
17. A compound according to claim 10 having the formula (3c):



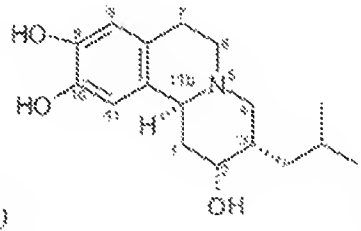
(3c)

and pharmaceutically acceptable salts thereof.

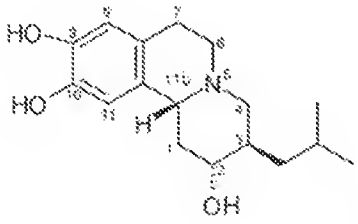
18. A compound according to claim 17 which is selected from the compounds (3c-I), (3c-II), (3c-III)and (3c-IV):



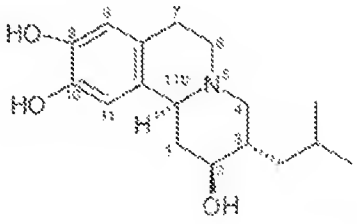
(3c-I)



(3c-II)



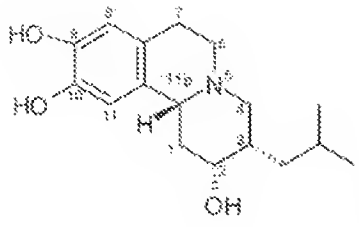
(3c-III)



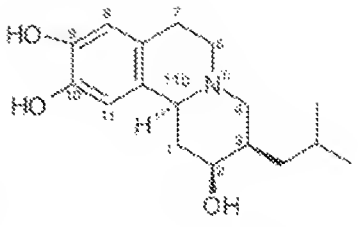
(3c-IV)

and pharmaceutically acceptable salts thereof.

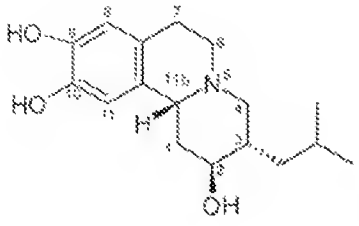
19. A compound according to claim 17 which is selected from the compounds (3c-V), (3c-VI) (3c-VII) and (3c-VIII):



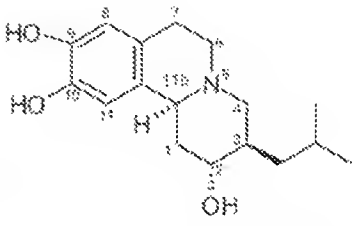
(3c-V)



(3c-VI)



(3c-VII)



(3c-VIII)

and pharmaceutically acceptable salts thereof.

20. A pharmaceutical composition comprising a compound as defined in any one of claims 2 to 19, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

21. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in medicine.

22. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of movement disorders.

23. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of Tourette's syndrome.

24. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of Huntington's disease.

25. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in halting or slowing the development of Huntington's disease.

26. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of inflammatory diseases.

27. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of multiple sclerosis.

28. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of psychoses.

29. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of schizophrenia.

30. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of a cognitive deficit associated with schizophrenia.

31. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in treating anxiety.

32. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of depression.

33. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for treating a cognitive deficit in a patient.

34. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for treating dementia.

35. A compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in treating asthma.

36. A compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for use in the treatment of diabetes mellitus.

37. The use of a compound according to any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for a use as defined in any one of claims 22 to 36.

38. A method of treating a disease or condition as defined in any one of claims 22 to 36, which method comprises administering to a patient in need thereof

a therapeutically effective amount of a compound as defined in any one of claims 1 to 20, or a pharmaceutically acceptable salt thereof.

Application No:	GB0816372.7	**Examiner:**	Dr Annabel Ovens
Claims searched:	1-38	**Date of search:**	21 January 2009

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	2 at least	WO 2007/130365 A (THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA) see compound 3 in Scheme 2
X	2 at least	Nuclear Medicine and Biology Vol. 33, No. 6, 2006, pages 685-694 Rajesh Goswami et al., "Fluoroalkyl derivatives of dihydrotetrabenazine as positron emission tomography imaging agents targeting vesicular monoamine transporters", see compound 3 in Schemes 1 and 2
X	2 at least	Nuclear Medicine and Biology Vol. 24, No. 2, 1997, pages 197-199 Douglas M Jewett et al., "A simple synthesis of [^{11}C]dihydrotetrabenazine (DTBZ)", see compound I in Fig. 1
X	2 at least	Nuclear Medicine and Biology Vol. 21, No. 2, pages 151-156 Jean N Dasilva et al., "Characterization of [^{11}C]tetrabenazine as an in vivo radioligand for the vesicular monoamine transporter", see OH-TBZ in Fig. 1
X	2 at least	Helvetica Chimica Acta Vol. 41, Issue 6, 1958, pages 1793-1806 A Brossi et al., "Syntheses in the emetine series. III. 2-Hydroxyhydrobenzo[a]quinolizine", see compound VIIa at page 1795
X	2 at least	Helvetica Chimica Acta Vol. 41, Issue 1, 1958, pages 119-139 A Brossi et al., "Synthesis in the emetine series. I. 2-Oxohydrobenzo[a]quinolizines", see compound XLIV at page 127
A	-	Chirality Vol. 9, Issue 1, 1997, pages 59-62 Michael R Kilbourn et al., "Absolute configuration of (+)-α-dihydrotetrabenazine, an active metabolite of tetrabenazine"

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

A61K; C07D

The following online and other databases have been used in the preparation of this search report

CAS-ONLINE, EPODOC, WPI

International Classification:

Subclass	Subgroup	Valid From
C07D	0455/06	01/01/2006
A61K	0031/4745	01/01/2006
A61P	0003/10	01/01/2006
A61P	0011/06	01/01/2006
A61P	0025/14	01/01/2006
A61P	0025/18	01/01/2006
A61P	0025/22	01/01/2006
A61P	0025/24	01/01/2006
A61P	0025/28	01/01/2006
A61P	0029/00	01/01/2006
A61P	0037/00	01/01/2006